Medical
Fapron

The Journal of Allergy

VOL. 20 JANUARY, 1949 NO. 1

Original Articles

QUANTITATIVE IMMUNOLOGIC STUDIES WITH ALLERGENS*†

I. COLORIMETRIC ABSOLUTE METHODS IN THE ANALYSIS OF FACTORS INFLUENCING PRECIPITATION OF RAGWEED POLLEN EXTRACTS AND HOMOLOGOUS RABBIT ANTISERA

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ANALYSIS of the immunologic mechanisms operative in pollinosis has, to date, been severely limited by the failure to isolate and identify the chemical substances responsible for the specific antigenic activity of pollen extracts. Despite this limitation, however, a number of provocative immunologic observations have been made. Recent interest has been devoted particularly to the role of "blocking antibody," first observed by Cooke,[1] and later studied intensively by Loveless[2, 3] and others,[4-12] in relation to the clinical response of hay fever subjects receiving prophylactic injections of pollen extracts.

While these investigators agree that blocking antibody appears in the blood stream of treated subjects, there has been no agreement upon the correlation of the antibody level and clinical relief. At least part of the disagreement results from the relative inaccuracy of the skin-neutralizing techniques of titration employed by the majority of investigators. Attempting to overcome this deficiency, Hampton and associates[13] developed the use of a precipitin technique and Johnson and co-workers[14] refined the method to titrate blocking antibody by measuring the volume of precipitates obtained from a standard dose of rabbit antiragweed serum by varying amounts of ragweed extract in the presence of human serum. A recent study of sixty-one patients employing this in vitro method revealed a general lack of correlation between blocking antibody titer and degree of clinical protection.[15] The volumes of precipitate measured in this procedure may not, however, be perfectly correlated with absolute amounts of precipitate. Accordingly, a quantitative absolute precipitin technique[16] has, in the present investigation, been adapted to the measurement of precipitates obtained by the interaction of whole ragweed extracts and antiragweed rabbit serum. Our first studies measured the absolute amounts of

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†This investigation was supported by a grant from the John and Mary R. Markle Foundation.

nitrogen precipitable from a standard rabbit antiragweed serum by increasing amounts of ragweed extract and established a curve for this relationship throughout the reaction range, particularly in the zone of antibody excess. This was followed by several quantitative studies of the dynamics of the interrelationship of antigen, ragweed antibody, and blocking antibody. Certain irregularities in the behavior of plain ragweed extracts during storage necessitated detailed studies of this phenomenon before proceeding with attempts to measure the blocking antibody.

The present report deals with the techniques employed and preliminary studies of some of the factors influencing precipitation of rabbit antiragweed serum by ragweed extracts. A separate report[17] is concerned with the deterioration of extracts under various conditions of storage, its prevention by glycerin, and the parallel decline during storage of the skin-sensitizing and precipitinogen activity of ragweed extracts.

MATERIALS AND METHOD

A. *Ragweed Extracts.*—All extracts were prepared from defatted commercial low ragweed pollen, using Coca's fluid as the extracting medium. Several different extracts were used during the course of the study; the earliest experiments used lot #C-126-46, which had been lyophilized several months earlier. A fresh ampule was rehydrated before its employment in each major experiment and a moderate amount of sediment removed by centrifugation, which yielded a clear supernatant fluid containing 0.194 mg. protein nitrogen per milliliter. This quantity compared favorably with the concentration of the extract before lyophilization (0.20 mg.).

After supplies of lyophilized extract had been exhausted, fresh extracts were made. Lot 46 F was prepared on June 26, 1946, and the entire batch of filtered extract was stored in 30 c.c. vials in the deep freeze at –20° centigrade. This was done on the assumption at that time that storage in the frozen state would preserve activity optimally. A new vial of frozen extract was thawed before use and the sediment which always formed on thawing was discarded after centrifugation and aspiration of the clear supernatant fluid. Lot 72146 was prepared by extraction of 8.0 Gm. of pollen per 100 milliliters extracting fluid, instead of the 5.0 Gm. per 100 milliliters usually employed. Lot 111046 was prepared with the aid of the Waring blender and was then stored in the deep freeze.

Three and 5 per cent extracts were simultaneously prepared from the same lot of dried pollen on June 17, 1946. These were used in studies of the relationship between concentration of extracting mixtures and the protein-nitrogen concentration of extracts obtained, as well as of the relation between protein-nitrogen units and precipitating activity.

B. *Rabbit Antiragweed Sera.*—A number of rabbits were available which had been immunized with low ragweed extracts over a period of several years as described in earlier communications from this laboratory.[13, 14] They were injected intramuscularly with 1 to 1.5 million units of ragweed pollen and aluminum hydroxide cream at multiple intramuscular sites, and bled by cardiac

puncture twenty-one days later. The serum was obtained by centrifugation, aseptically, pooled, and stored in the frozen state. Several pools were prepared from the same group of rabbits, following separate booster inoculations. These were designated 45, 46A, and 46B.

C. *Technique of the Quantitative Precipitin Reaction.*—All steps in this procedure were carried out aseptically to avoid any possible contribution of bacterial growth to the nitrogen analyses of the specific precipitates. All centrifugations were at 2,500 R.P.M. in an angle head in a size II International Centrifuge kept in the cold room. A sufficient quantity of the antigen was either rehydrated or thawed, the turbid solutions centrifuged, and the clear supernatant fluid obtained by aspiration. In earlier experiments, ragweed extracts were diluted with physiologic saline to yield the following concentrations of protein nitrogen units (P.N.U.) per milliliter: 10,000, 5,000, 2,500, 1,250, 625, and 312. At the same time, a sufficient quantity of the rabbit antiragweed serum was thawed, clarified by centrifugation and diluted 1:4 with sterile saline.

The precipitin reactions were carried out in sterile Pyrex Wassermann tubes calibrated at a 2.5 ml. volume. All tubes were prepared in duplicate, the precipitate nitrogen obtained being determined as the average of the duplicate analyses. Two milliliters of each of the diluted extracts were pipetted into corresponding pairs of tubes, and 2 ml. of the 1:4 dilution of rabbit antiragweed serum added to each tube. Controls, in duplicate, containing, respectively, serum alone and antigen alone, were established at the same time (Table I). The contents of each tube were thoroughly mixed by rotating them individually. All tubes were then incubated in a water bath at 37° C. for one hour after which they were refrigerated for forty-four to forty-eight hours.

At the end of this time, all tubes were centrifuged at 2,500 R.P.M. for thirty minutes. They were then maintained in an ice water bath while the clear supernatant fluids were withdrawn, taking great care not to disturb the precipitate. A small amount of chilled saline was added to the sediment which was then loosened by agitation. A total of 3.0 ml. of chilled saline was added to each tube and the sediment carefully suspended in this volume. The centrifugation was repeated and the precipitate washed with a second 3.0 ml. volume of chilled saline in the same manner.

The washed precipitates were then dissolved by agitation after the addition of 0.3 ml. of N/10 NaOH to each tube. After solution of the precipitate, distilled water was added to bring the volume to the 2.5 ml. mark. Two milliliters quantities from each tube were transferred to separate 50 ml. Erlenmeyer flasks and 6.0 ml. of 12.5 per cent Na_2CO_3 added, followed by 1.0 ml. of 0.1 per cent $CuSO_4$. All flasks were allowed to stand one hour at room temperature after which 1.0 ml. of freshly diluted 1:3 Folin-Ciocalteau reagent was added to each. The contents were mixed thoroughly, allowed to stand twenty-five minutes at room temperature, and then transferred to colorimeter tubes for determination of per cent transmission (%T) in a balanced cell photoelectric colorimeter, using a Corning #3480 filter (Hr Red Shade Yellow; maximum transmission equal to 610 millimicrons, transmits less than 20 per cent below 570 millimicrons).

TABLE I. PRECIPITATION OF

TUBE #	1	2	3	4	5	6	7	8
Antigen P.N.U./ml.	312		625		1,250		2,500	
Ml. Antigen	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Ml. Saline	—	—	—	—	—	—	—	—
Ml. ARRS 45A 1:4	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Per cent Transmission	82.75	83.25	76.25	75.75	70.25	71.75	63.75	62.25
O.D.	.0822	.0796	.1177	.1206	.1534	.1442	.1956	.2059
Average O.D.	.0809		.1192		.1488		.2008	
O.D. subtracted*	.0160		.0165		.0170		.0177	
Corrected O.D.	.0649		.1027		.1318		.1831	

Protocol 26, performed on Aug. 21, 1946, when the antigen was 31 days old.
*O.D. of serum control plus appropriate proportion of antigen control.

The per cent transmission was converted to optical density (O.D.) by reference to a conversion table. The average optical density was corrected by subtraction of the optical density attributable to the serum and antigen controls.

Observations on the Interaction of Ragweed Extracts and Rabbit Antiragweed Serum.—

A. *Determination of Relation Between Nitrogen Content of Precipitates and the Optical Density of Appropriate Folin-Ciocalteau Reagent Colors*: The colorimetric procedure was first applied to a solution of Hammersten's casein, standardized by Kjeldahl analysis. A curve was constructed after colorimetric analysis of a number of dilutions prepared from this standard solution. For casein, a factor of 0.065 optical density was found to represent 0.01 mg. nitrogen. The analysis of the factor for specific ragweed-antiragweed precipitates yielded somewhat more variable results but the order of magnitude was similar. For these latter determinations, 10 to 40 ml. of the rabbit antiragweed serum were precipitated with an equal volume of 10,000 P.N.U. per milliliter ragweed extract. The precipitate was obtained, washed, and dissolved in the usual manner, and the resulting solution divided into two portions, one of which was analyzed for nitrogen by the Kjeldahl method while the other was diluted serially and the color values of the various solutions then obtained. The optical densities so determined were then plotted against the known nitrogen values. Several different rabbit sera were precipitated in this fashion with several different antigens, and a single serum was precipitated with various amounts of the same antigen. The value obtained with lyophilized antigen C-126-46 was 0.0435, optical density (O.D.) = 0.01 mg. nitrogen. We never again obtained so low a factor. Lyophilized extracts, however, were not used in subsequent experiments. The factors thereafter ranged from 0.075 to 0.1, O.D. = 0.01 mg. nitrogen. Because of the variability in results obtained in these titrations, we recorded all results of titrations in terms of optical density rather than in actual micrograms of nitrogen. For the calculation of micrograms of nitrogen, however, the factor of 0.085, O.D. = 0.01 mg. nitrogen may be used, since this figure represents the average of the several determinations made with nonlyophilized antigens.

Experiments performed with serums 45A and 46A will illustrate the method (Table II).

45A SERUM BY 7/21/46 ANTIGEN

9	10	11	12	13	14	15	16	17	18
5,000		10,000		20,000		Antigen Control 20,000		Serum Control	
2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	—	—
—	—	—	—	—	—	2.0	2.0	2.0	2.0
2.0	2.0	2.0	2.0	2.0	2.0	—	—	2.0	2.0
57.5	56.0	53.5	51.0	59.0	59.25	97.0	97.0	96.25	96.5
.2403	.2518	.2716	.2924	.2291	.2273	.0132	.0132	.0166	.0155
.2461		.2820		.2282		.0132		.0161	
.0190		.0230		.0290					
.2271		.2590		.1992					

1. 30 c.c. ARRS 46A, 1:4 dilution, were mixed with 30 c.c. of the 7/21/46 antigen, 5,000 P.N.U. per milliliter, and
2. 40 c.c. ARRS 45A, 1:4 dilution, were mixed with 40 c.c. of the 7/21/46 antigen, 10,000 P.N.U. per milliliter.

The mixtures were incubated for one hour at 37°C. and then refrigerated for sixty hours after which the precipitates obtained were washed twice with chilled saline, quantitatively combined after solution with N/10 NaOH, and each solution of precipitated antibody was made up to 10.0 ml. in volume. Kjeldahl determinations were then made on duplicate 3.5 ml. volumes of each solution of antibody. The 45A serum yielded 0.640 mg. of nitrogen precipitate from the 10 ml. originally used (40 ml. of a 1:4 dilution) or a yield of precipitate nitrogen of 0.064 mg. per milliliter. Similar calculation indicated that 46A serum yielded 0.082 mg. nitrogen per milliliter.

The remainder of the solutions of the precipitates obtained from 46A and 45A serums was then diluted with saline to contain .025 mg. of nitrogen per milliliter, from which further dilutions were made as indicated in Table II.

TABLE II. RELATION BETWEEN NITROGEN CONTENT AND FOLIN COLOR REACTIONS OF PRECIPITATES OBTAINED FROM 45A AND 46A RABBIT ANTIRAGWEED SERUM BY 7/21/46 LOW RAGWEED EXTRACT

SOLUTION	TUBE #	ML. TAKEN	ML. WATER	GAMMAS N	% T	O.D.	AV. O.D.
45A*	1	1.0	1.0	25	64.5	.1905	.1922
	2	1.0	1.0		64.0	.1939	
	3	0.8	1.2	20	69.5	.1580	.1580
	4	0.8	1.2		69.5	.1580	
	5	0.6	1.4	15	76.25	.1177	.1177
	6	0.6	1.4		76.25	.1177	
	7	0.4	1.6	10	83.5	.0783	.0770
	8	0.4	1.6		84.0	.0757	
46A*	1	1.0	1.0	25	64.25	.1922	.1889
	2	1.0	1.0		65.25	.1855	
	3	0.8	1.2	20	70.25	.1534	.1557
	4	0.8	1.2		69.50	.1580	
	5	0.6	1.4	15	77.0	.1135	.1164
	6	0.6	1.4		76.0	.1192	
	7	0.4	1.6	10	83.25	.0796	.0790
	8	0.4	1.6		83.5	.0783	

*Concentration of .025 mg. nitrogen per milliliter.
% T = Per cent transmission
O.D. = Optical density
Av. O.D. = Average optical density

Typical colorimetric determinations were made in duplicate and the resultant optical densities plotted against the determined nitrogen values.

This experiment also demonstrates that identical color projection curves are obtained with precipitates obtained from two different lots of rabbit antiragweed serum. Later studies revealed that similarly identical color values were obtained for precipitates obtained from a single rabbit serum after precipitation by different quantities of the same antigen. This indicates that significant differences of the antigen-antibody ratios probably present in such precipitates do not affect the color values obtained per unit of nitrogen.

B. *Precipitation of Ragweed Antiragweed Serum by Ragweed Extract*:

1. The Curve of Precipitation.—A typical protocol is illustrated in Table I which records the results obtained when serum 45A was precipitated with increasing amounts of a thirty-day old 5 per cent ragweed extract (7/21/46). The amount of precipitate obtained increased as the antigen was increased, up to 5,000 P.N.U. per milliliter beyond which there was inhibition of precipitation. The configuration of the curve (Fig. 1) is like that which has been described for other precipitating systems.

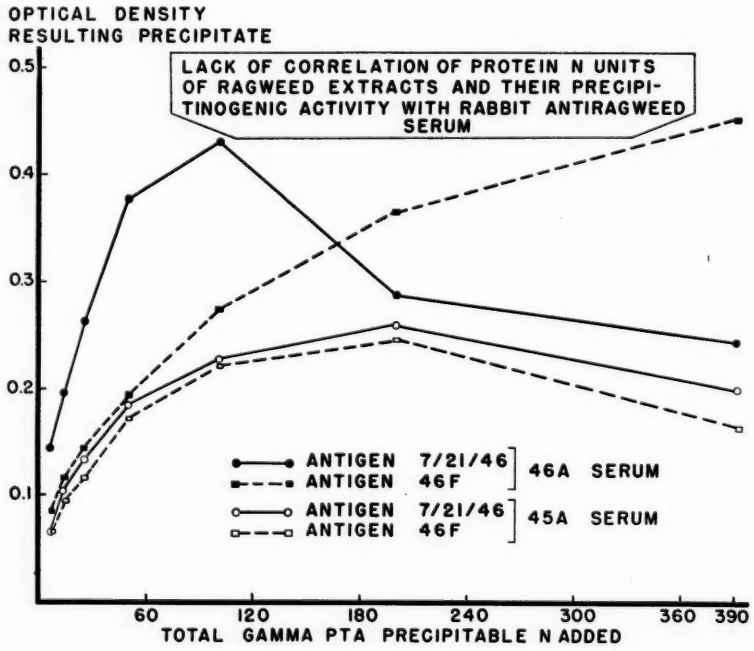

Fig. 1.

2. Effect of Duration of Refrigeration Upon Degree of Precipitation.—This experiment was performed in order to determine whether the forty-eight hour period of refrigeration ordinarily employed in quantitative precipitin studies with animal sera was sufficiently long for studies with the ragweed system. Duplicate sets of tubes were prepared as indicated in Table I, employing lyophilized extract C-126-46 and rabbit serum 45A. One set of tubes

was analyzed in duplicate after forty-eight hours of refrigeration, the second set after 168 hours. The corrected average optical densities observed for the corresponding pairs of tubes are indicated in Table III. The results obtained are practically identical, indicating that the forty-eight hour period of refrigeration suffices to carry the precipitin reaction to completion. The duplicability of the determination is incidentally revealed.

TABLE III. EFFECT OF DURATION OF REFRIGERATION ON DEGREE OF PRECIPITATION

	ANTIGEN (12/6/46) P.N.U./ML.						
	312	625	1,250	2,500	5,000	10,000	20,000
48 Hours	.109	.129	.154	.209	.255	.243	.235
168 Hours	.105	.133	.168	.202	.273	.253	.239

Figures represent corrected average optical densities.

3. RELATION BETWEEN PROTEIN NITROGEN UNITS AND PRECIPITINOGENIC ACTIVITY OF RAGWEED EXTRACTS.—One of the objectives of the present study was to determine whether there was strict correlation between the protein nitrogen content and precipitinogenic activity of various ragweed extracts. Extracts at 3 and 5 per cent concentrations were prepared simultaneously from the same lot of ragweed pollen. Upon analysis for phosphotungstic acid precipitable nitrogen, the 3 per cent extract was found to contain 0.19 mg. nitrogen per milliliter and the 5 per cent extract 0.33 mg. nitrogen per milliliter.

A parallel series of dilutions was then made with each extract and curves of precipitation obtained for each with the same serum, at the same time. The results of this analysis (Table IV) reveal definite differences in the amount of precipitation obtained with equal amounts of protein nitrogen of the two extracts. The 5 per cent extract apparently precipitated twice as much total nitrogen per unit of antigen protein nitrogen, the differences between the two extracts being more evident in the region of considerable antibody excess. Unsuccessful attempts were made to demonstrate similar differences in the two extracts by direct skin testing of ragweed sensitive subjects. Much more detailed studies by neutralization of passive transfer sites were made later with extracts in various stages of deterioration.[17]

TABLE IV. PRECIPITATION OF ANTIRAGWEED SERUM BY IDENTICAL AMOUNTS OF PROTEIN NITROGEN FROM 3 AND 5 PER CENT RAGWEED EXTRACTS

	P.N.U./ML. ANTIGEN						
	312	625	1,250	2,500	5,000	10,000	20,000
3 Per Cent Extract	.0687	.0972	.1370	.1768	.2522	.2061	.1871
5 Per Cent Extract	.1007	.1333	.1716	.2070	.2635	.2177	.1895

Figures represent corrected average optical densities.

Striking differences in the relation between protein nitrogen content and total nitrogen precipitated were manifested by two different ragweed extracts and a single rabbit serum. Antigen 46F (47 days old) and 7/21/46 (23 days old) were titrated against serum 46A in the same protocol. The results of this experiment appear in Fig. 1. Equal amounts of phosphotungstic acid precipitable antigen nitrogen of extract 7/21/46, precipitated considerably more total nitrogen than did extract 46F and the slopes of the two precipitation curves were quite different. It will be noted, however, that the maximal amount of nitrogen precipitable by the two extracts was the same, four times as much

antigen protein nitrogen of extract 46F being required to achieve this degree of precipitation. As our later studies revealed, variability in the rate of deterioration of plain ragweed extracts under storage in the deep freeze has an important influence upon precipitinogenic activity of the extracts, independently of the effect on antigen protein nitrogen. That this is not the only factor operative is indicated by additional experiments in which antigens 46F and 7/21/46 were employed in the precipitation of serum 45A. These experiments were performed at different times, with 46F when that antigen was 17 days old and 7/21/46 when it was 31 days old. The results are also plotted in Fig. 1. It is evident that 46F and 7/21/46 antigens are qualitatively different in their precipitation of 46A serum. It is also evident that 45A and 46A serums are qualitatively different when precipitated with the same antigen (7/21/46, 31 days old when precipitating 45A and 23 days old when precipitating 46A serum). Nevertheless, 46F and 7/21/46 antigens appear to be qualitatively and quantitatively alike in the precipitation of 45A serum. We have no satisfactory explanation as yet for these striking immunologic differences.

4. Evidence of Deterioration of Ragweed Extracts During Storage in the Deep Freeze.—Extract 7/21/46, which had been maintained in the frozen state, was used in a number of experiments with serum 46A. Experiments performed with this antigen when it was 52 days old yielded unexpectedly small amounts of precipitate nitrogen per unit of protein antigen nitrogen. Repetition of the titration when the antigen was 71 days old confirmed the observation, while the protein nitrogen content of the extract was found unchanged. Table V and Fig. 2 illustrate the magnitude of the change in precipitation observed with increasing extract age.

The possibility that freezing alone was responsible for part of this deterioration was investigated by preparing a fresh ragweed extract, freezing and thawing one-half of it once only, then titrating the once-frozen and the fresh,

Table V. Precipitation of 46A Serum by Variously Aged Frozen-Stored Extract 7/21/46

Protocol No.	Date	Extract Age Days	Units of Antigen (2.0 c.c.) 625	1,250	2,500
23*	8/7	17	.1571	.2301	.3416
24†	8/13	23	.1960	.2605	.3768
29*	9/11	52	.1231	.1399	.1886
30†	9/30	71	.1098	.1406	.1805

Figures represent corrected average optical densities.
*No incubation during precipitation.
†Incubation 1 hour at 37° C. during precipitation.

Table VI. Precipitation of 46A Serum by Freshly Prepared and Once Frozen Extract 11/10/46*

	Units of Antigen (11/10/46) 2.0 ml. 156	312	625	1,250	5,000	10,000	20,000
Unfrozen Extract	.0941	.1402	.1880	.2521	.4790	.2859	.2761
After One Freeze Thaw		.0823	.1163	.1394	.2013	.2144	

Figures represent corrected average optical densities.
*Frozen at −70° C. in the CO_2 box.

untreated extract, against the same serum in the same protocol. Our first observation, recorded in Table VI, revealed a marked immediate drop in the precipitating activity of the frozen-thawed aqueous extract (with no further observed drop during the next twelve months of frozen storage). Other extracts, subjected to similar storage, resisted immediate deterioration but exhibited progressive deterioration during such storage. Corresponding glycerinated extracts, however, showed relatively much less loss of activity.[17]

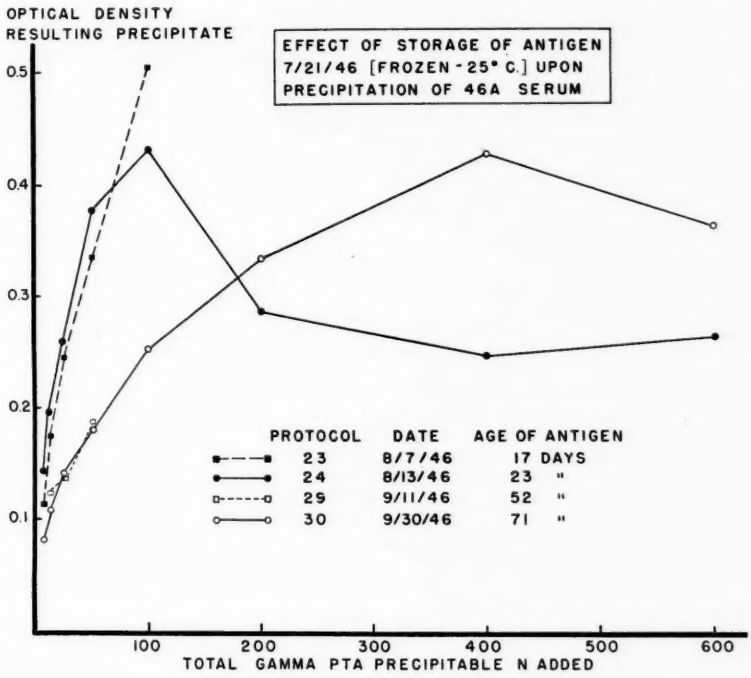

Fig. 2.

5. Effect of Incubation and Duration of Refrigeration on the Precipitation of Rabbit Antiragweed Serum 46A by 7/21/46 Extract.—Experiments on the effect of duration of refrigeration had been carried out earlier (B. 2 above). The early experiment, however, simply served to establish the forty-eight hour period as adequate to permit maximal precipitation, but only compared the forty-eight hour period with one of 168 hours. The present experiment was designed to determine the effect of centrifugation, (1) immediately after mixing, (2) after overnight refrigeration, and (3) after forty-eight hour refrigeration of mixtures of 7/21/46 extract and 1:4 dilution of 46A serum. Duplicate sets of tubes were analyzed under the above conditions; (a) without incubation, and (b) following one-hour incubation at 37°C. Thus, six sets of duplicate tubes were set up in this protocol, using 2.0 ml. of solutions of 7/21/46 extract containing 312, 625, 1,250, 2,500, and 5,000 P.N.U. antigen per milliliter, respectively, with 2.0 ml. of 1:4 serum 46A. Results of the analyses of precipitates obtained are given in Table VII.

TABLE VII. EFFECT OF INCUBATION AND DURATION OF REFRIGERATION ON PRECIPITATION OF 46A SERUM BY 7/21/46 EXTRACT

		ANTIGEN P.N.U./ML.				
		312	625	1,250	2,500	5,000
Not Incubated	Centrifuged Immediately	.0431 (37%)	.0966 (61.5%)	.1702 (74%)	.2437 (71.5%)	.3711 (80.5%)
	After Refrigeration Overnight	.0942 (81%)	.1409 (89.5%)	.2003 (87%)	.2991 (87.8%)	.3998 (86.5%)
	After 48 Hours' Refrigeration	.1164 (100%)	.1571 (100%)	.2301 (100%)	.3416 (100%)	.4622 (100%)
1 Hr. at 37° C. Incubated	Centrifuged Immediately	.0296 (25.8%)	.0841 (48.0%)	.1687 (68.0%)	.2920 (86.6%)	.4059 (80.7%)
	After Refrigeration Overnight	.1015 (89%)	.1517 (86.5%)	.2172 (88%)	.3128 (92.8%)	.4405 (87.5%)
	After 48 Hours' Refrigeration	.1143 (100%)	.1757 (100%)	.2468 (100%)	.3373 (100%)	.5055 (100%)

Figures represent corrected average optical densities.
Figures in parentheses represent percentage of maximal precipitation.

This experiment clearly demonstrates that on immediate centrifugation, whether mixtures are incubated or not, a progressively higher per cent of the total precipitable nitrogen obtainable is precipitated as the antigen concentration is increased. Conversely, in the region of considerable antibody excess (312 P.N.U.) only a small percentage of the total precipitable nitrogen appears after immediate centrifugation (25 to 37 per cent). This would suggest that the avidity of ragweed antigen for rabbit antiragweed antibody is least in the region of considerable antibody excess and that one should therefore select this range for maximal sensitivity in detecting thermostable antibody. The total amount of precipitate obtained on immediate centrifugation with the smaller quantities of antigen is, however, too small for necessary accuracy. Increasing the concentration of antigen, or refrigerating overnight before centrifugation, diminishes the percentile differences in precipitation by low antigen concentrations compared with the higher concentrations. The differences encountered, however, suggest that experiments designed to establish maximal sensitivity of detection of blocking antibody may have to incorporate variable antigen-antibody ratios as well as differing refrigeration times.

Observations on the Effect of "Blocking Antibody".—

A. We have already indicated that quantitative studies of "blocking antibody," although the original goal of this series of investigations, were interrupted by the necessity to investigate deterioration of antigen. A few preliminary observations of some interest have been made, which merit description at the present time.

Early in the course of the study, fasting serums were obtained from a nonatopic individual (SCB) and from a treated ragweed sensitive subject (MCJ). An experiment was set up as illustrated in Table VIII. A considerable inhibition of precipitation was noted in the presence of 0.5 c.c. of MCJ serum while there appeared to be slight increase of precipitation in the presence of 1.0 ml. of the nonatopic (SCB) serum. This preliminary experiment encouraged the belief that these methods would lend themselves satisfactorily to the quantitative determination of blocking antibody.

B. *The Effect of Varying Amounts of Serum Containing Blocking Antibody Upon the Precipitation of a Constant Quantity of Rabbit Antiragweed Serum and Whole Ragweed Extract; Influence of Diluent*: The experiment illustrated in Table VIII indicated that it would be possible to employ as much as 1.0 ml.

TABLE VIII. EFFECT OF SERUM FROM NORMAL (SCB) AND TREATED RAGWEED SENSITIVE SUBJECT (MCJ) ON PRECIPITATION OF RABBIT ANTIRAGWEED SERUM BY WHOLE RAGWEED EXTRACT

	CONTROL		SCB SERUM		MCJ SERUM*	
	1	2	3	4	5	6
ARRS 45A 1:4	2.0	2.0	2.0	2.0	2.0	2.0
5,000 units/ml. antigen†	2.0	2.0	2.0	2.0	2.0	2.0
SCB serum‡	—	—	1.0	1.0	—	—
MCJ serum‡	—	—	—	—	0.5	0.5
Saline	1.0	1.0	—	—	0.5	0.5
Per cent transmission	56.00	59.75	53.5	51.75	87.25	86.5
O.D.	.2518	.2236	.2716	.2861	.0593	.0630
Av. O.D.	.2377		.2788		.0612	

*On perennial treatment, last injection of 10,000 P.N.U. antigen four weeks earlier.
†Extract 1/26/46.
‡Heated to 60° C. for one hour before use.

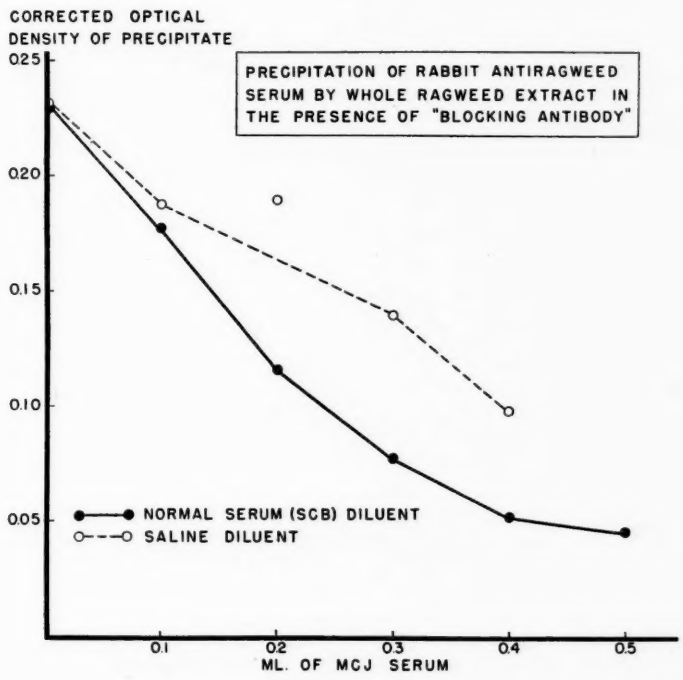

Fig. 3.

of serum in measurements of blocking antibody with the selected 2.0 ml. quantities of rabbit antiragweed serum and whole ragweed extract, since a normal serum (SCB), in that amount, failed to inhibit precipitation. The following experiment was performed to determine the inhibition of precipitation by quantities of MCJ serum ranging from 0.1 to 0.5 ml., in a total volume of 1.0 milliliter.

The first study was made with SCB serum as diluent, a sufficient amount of this noninhibiting serum being added to bring the combined volume of the two serums to 1.0 milliliter. Thus, 0.9 ml. SCB serum was mixed with 0.1 ml. MCJ; 0.8 ml. SCB, with 0.2 ml. MCJ, et cetera. An addition of 2.0 ml. of 5,000 P.N.U. per milliliter of antigen was made to 1.0 ml. of the corresponding serum mixture in duplicate, and allowed to stand at refrigerator temperature for four hours. At the end of this time, 2.0 ml. of the ARRS 1:4 were added, with mixing, incubated at 37°C. for one hour, refrigerated forty-eight hours, then analyzed as usual.

One week later, the study was repeated with similar quantities of MCJ serum diluted in saline instead of in SCB serum. The results of these studies are plotted in Fig. 3. The increasing inhibition of precipitation with increasing quantities of MCJ serum, diluted either in normal serum or in saline, is notable. The inhibitory effect of MCJ serum is definitely less, however, in the presence of saline as diluent; the differences observed are significant in view of the fact that equal precipitation was observed in the two experiments in the absence of any serum (O.D. = 0.233).

This observation, contrary to the earlier experience with the same normal serum (Table VIII), strongly suggested that normal serum might inhibit the precipitation of rabbit antiragweed serum by whole ragweed extract. Additional experiments have since confirmed that suspicion, so that it will be necessary to study a large number of "normal" sera obtained under a variety of circumstances before proceeding to the analysis of sera from treated ragweed sensitive subjects.

C. *Effect of the Preincubationary Period of Contact Between Antigen and Human Serum and the Duration of Refrigeration After Addition of Rabbit Antiragweed Serum Upon the Degree of Precipitation Obtained*: The duration of preincubationary contact between whole ragweed extract and human serum under investigation for blocking antibody content seemed to have been rather arbitrarily determined. We thought it of some importance to attempt to investigate the dynamics of the interaction among ragweed antigen, blocking antibody, and rabbit antiragweed antibody. It was desirable to determine this accurately, both from the purely theoretical point of view and also as a prerequisite to the establishment of optimal conditions for the quantitative estimation of blocking antibody. Accordingly, 2.0 ml. of 5,000 units per milliliter ragweed extract were mixed, in duplicate, with 0.5 ml. of a normal human serum (SCB). Six pairs of tubes were so established, with appropriate controls in each instance. An addition of 2.0 ml. of ARRS 45A was made to two pairs of tubes immediately. These were incubated for one hour at 37°C., and then refrigerated. One pair of tubes was analyzed for precipitate nitrogen after forty-eight hours of refrigeration; the corresponding pair was analyzed after ninety-six hours of refrigeration.

The remaining four pairs of tubes were refrigerated. At the end of four hours, ARRS 45A was added to two pairs of tubes, which were then processed as above, and at the end of eighteen hours, the remaining two pairs of tubes were similarly treated.

An identical experiment was performed one week later, using 0.5 c.c. of MCJ serum instead of normal human serum. The results of these analyses are summarized in Table IX. It will be noted that there is consistent diminution of precipitation in the presence of 0.5 c.c. of normal, heat-inactivated human serum. The diminution of precipitation observed with normal human serum is apparently not affected by duration of contact between serum and antigen preceding addition of ARRS, or by duration of refrigeration after addition of ARRS. It is of incidental interest that the values for precipitate nitrogen obtained in six pairs of determinations in the absence of human serum show a maximum discrepancy of 8.5 per cent. The corresponding discrepancy in the presence of 0.5 c.c. of normal human serum was 12.5 per cent. The mean per cent precipitation in the presence of 0.5 c.c. normal human serum was 87.5.

TABLE IX. EFFECT OF DURATION OF CONTACT BETWEEN RAGWEED ANTIGEN AND HUMAN SERUM (NORMAL AND TREATED RAGWEED) AND OF LENGTH OF REFRIGERATION UPON PRECIPITATION OF ANTIRAGWEED RABBIT ANTIBODY

DURATION OF PRE-INCUBATIONARY CONTACT BETWEEN ANTIGEN AND HUMAN SERUM	NORMAL SERUM (SCB)				TREATED RAGWEED (MCJ)			
	48-HOUR REFRIGERATION		96-HOUR REFRIGERATION		48-HOUR REFRIGERATION		96-HOUR REFRIGERATION	
	CONTROL*	SERUM†	CONTROL	SERUM	CONTROL*	SERUM†	CONTROL	SERUM
Immediate	.2672	.2348	.2559	.2123	.2655	.1340	.2518	.1612
	88%‡		83%		50%‡		64%	
4 hours	.2516	.2337	.2720	.2228	.2704	.1232	.2559	.1192
	92.6%		82%		45.5%		46.6%	
18 hours	.2696	.2437	.2742	.2421	.2372	.0815	.2462	.0726
	93.3%		88%		34.2%		29.6%	

*Corrected average optical densities obtained in absence of any human serum.
†Corrected average optical densities obtained in presence of 0.5 c.c. of human serum.
‡$\frac{\text{O.D. Serum}}{\text{O.D. Control}} \times 100 = \%$ of maximum precipitation obtained in presence of human serum.

The effect of the serum from the treated ragweed sensitive subject was quite different. We observed progressive decrease in the precipitation of antiragweed rabbit serum as contact between ragweed antigen and human thermostable antibody was prolonged before addition of the antiragweed rabbit serum. This progressive decrease in precipitation with prolongation of contact, up to eighteen hours' maximum, was not eliminated by increasing the time of refrigeration to ninety-six hours.

As a result of this study, we decided, in the future, to employ an eighteen-hour period of preincubationary contact between ragweed extract and serums under investigation for blocking antibody content.

D. *Effect of Pooled Serum From a Treated Ragweed-Sensitive Subject (MCJ) Upon the Precipitation of Rabbit Antiragweed Antibody Throughout the Reaction Range*: This experiment was designed to investigate the relative intensity of inhibition exhibited by serum containing blocking antibody in the presence of changing ratios of ragweed extract to antiragweed antibody. The objective of the experiments was to select a reaction range which would permit maximum sensitivity in the measurement of blocking antibody.

For this study, we pooled 1.5 to 4.5 ml. quantities of fifteen serums obtained from a treated ragweed sensitive subject (MCJ) at weekly intervals during the

TABLE X. EFFECT OF HEAT INACTIVATED POOLED MCJ* SERUM UPON THE PRECIPITATION OF RABBIT ANTIRAGWEED ANTIBODY THROUGHOUT THE REACTION RANGE

	ANTIGEN UNITS PER MILLILITER							
	2,000	4,000	6,000	8,000	10,000	15,000	20,000	30,000
(a) In saline	.1121†	.1711	.1959	.2176	.2475	.2639	.3133	.3550
(b) In presence of 0.3 ml. MCJ serum	.0367	.0899	.1181	.1590	.1861	.2295	.2519	.3123
$\frac{(b)}{(a)} \times 100$‡	32.7	52.5	60.5	73.0	75.5	87.0	80.5	88.0

*Treated ragweed sensitive subject.
†Corrected average optical densities.
‡Per cent precipitated in presence of serum.

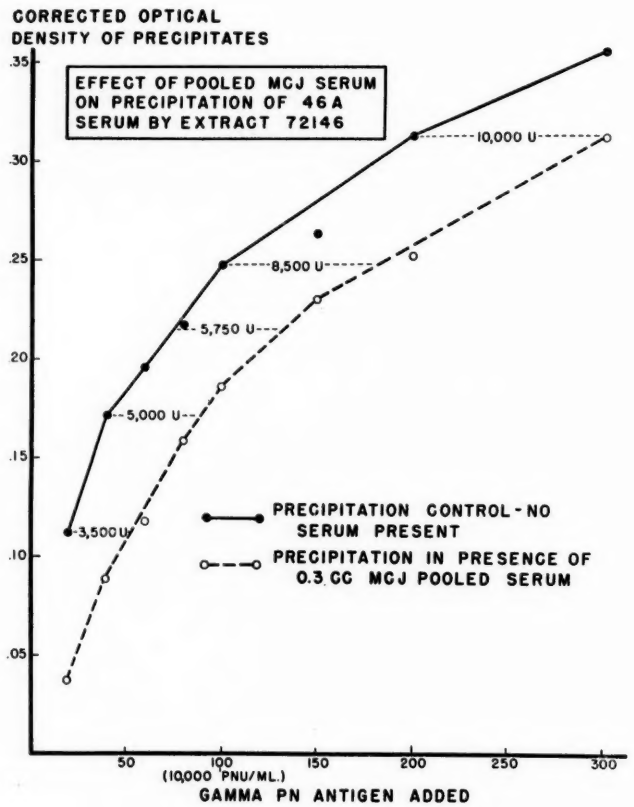

Fig. 4.

course of treatment. A parallel set of duplicate titrations was then established with 2.0 ml. of 1:4 dilution of 46A serum and 1.0 ml. of antigen in 2,000 to 30,000 units per milliliter concentration. To one set of tubes we added 1.0 ml. of saline and to the other, 0.3 ml. of the pooled serum (MCJ) heated at 60°C. for thirty minutes and 0.7 ml. of saline. The tubes and appropriate controls were then incubated, refrigerated, and analyzed in the usual way. The results of this study are summarized in Table X and Fig. 4.

As indicated in Fig. 4, there is a well-defined inhibition of precipitation in the presence of the human serum throughout the reaction range. The two curves follow each other very closely and at first glance there appears to be a symmetrical depression of precipitation. As revealed in Table X, however, there is relatively greater inhibition in the presence of 2,000 P.N.U. antigen per milliliter, than there is in the presence of 30,000 P.N.U. antigen per milliliter, there being 32.7 and 88 per cent of maximal precipitation at these concentrations, respectively. Furthermore, the relative inhibiting activity of the fixed amount of MCJ serum progressively diminished as the amount of antigen employed increased. This could be readily explained if the dose of serum used were capable of "neutralizing" a fixed, absolute amount of antigen. As indicated in Table X, and in discussion below, this does seem to be the case. There is, however, some tendency for more effective "blocking" activity with the larger doses of antigen, at which level some 10,000 units of antigen appear to be blocked (Fig. 4) instead of the average 4,000 units blocked at lower dosages.

DISCUSSION

The elimination of skin neutralization and the substitution of a convenient in vitro method for the measurement of blocking antibody is dependent upon a complete familiarity with and control of the reagents to be employed. The present experiments which are only preliminary steps in that direction have yielded some rather useful information. It is apparent that the colorimetric method of analysis of specific precipitates is an accurate procedure, having great reproducibility, so long as uniformly active extracts and antisera are employed. Despite the fact that complex, impure whole ragweed extracts were used as antigens, the characteristics of the precipitation between the extracts and pooled rabbit antiragweed sera were essentially the same as those which have been described for other rabbit antibody precipitations. The unexplained differences in behavior of the extracts and sera (Fig. 1), however, illustrate the difficulties involved in quantitative measurement of the various immunologically active substances in pollinosis so long as we are required to work with impure extracts. Cooke[18] has stressed the necessity for fractionation and purification of the ragweed antigen, an accomplishment which would provide for rapid increase in our understanding of the immunology of pollinosis. Nevertheless, the whole antigen extract can be used in attempts to measure blocking antibody if single lots of such extracts can be prepared and maintained relatively free from deterioration.

The observation, summarized in Table III, that a forty-eight-hour period of refrigeration suffices for maximal precipitation is also in keeping with the known behavior of other precipitating systems. The lack of parallelism between protein nitrogen unit and precipitinogenic activity of whole ragweed extracts (Table IV) is the subject of further discussion in the next communication as is the evidence that nonglycerinated extracts may deteriorate fairly rapidly under several storage conditions while glycerinated extracts resist such deteriorations.[17]

Table VII reveals that ragweed extract has an apparently greater avidity for rabbit antiragweed antibody in the region of considerable antibody excess.

This suggests that further studies of blocking antibody be conducted with rabbit antiragweed sera of high potency, so that adequate amounts of precipitate may be obtained in the presence of small amounts of antigen.

The observations on blocking antibody are quite fragmentary. It was, however, evident that inhibition of precipitation occurred in the presence of posttreatment serum of ragweed sensitive subjects and that a preliminary period of preincubationary contact between ragweed extract and human serum increased the inhibition. Table X and Fig. 4 demonstrate fairly clearly that the thermostable serum produced symmetrical depression of precipitation throughout the reaction range. There appears to be a fairly uniform blocking of approximately 4,000 units of antigen, making it seem that the "unblocked" residual antigen retains its precipitating power intact.

Such theoretical objections as may have existed with regard to accepting the duality of antibodies in pollinosis would seem to have been partly resolved by a number of recent investigations. Miller and Campbell[19] report that the nonprecipitating reagin of egg-sensitive individuals will specifically adsorb to an egg albumin rabbit antiegg-albumin precipitate. These findings are interpreted as demonstrating that antiegg reagin and antiegg precipitin may be different forms of the same antibody, the former being monovalent. Of more direct interest is the important series of papers by Heidelberger and co-workers.[20, 21] These studies reveal that the characteristics of antibodies produced by a given animal species to a protein antigen may vary with the route of administration and nature of the antigen. While studies were not performed with inhalation as the route of administration of antigen, we might speculate about the possibility that reagin develops in the "atopic" individual in response to the inhalation of natural pollen while subcutaneous injections produce an antibody with the characteristics of blocking antibody. The nonatopic individual may simply be responding to inhaled pollen with so little reagin development that it remains undetected; we know the nonatopic responds to subcutaneous injections of ragweed extract by producing small quantities of blocking antibody. It would be of interest to study the sera of more nonatopic individuals, especially with the aid of a simple globulin concentrating procedure (and shortly after the peak of the pollen season), in order to determine whether antiragweed reagins may be detected. Certain reagins to simple chemicals have now been produced in the experimental animal,[22] lending additional support to the concept that normal humans may also respond to the inhalation of pollen by producing reagin.

Finally, the recent observations of Rice[23] on antibody inhibition of complement-fixing antibody seem pertinent to the problem of blocking antibody in pollinosis. Rice has discovered that certain species of animals fail to produce complement-fixing antibody to some antigens but that the sera of such animals are capable of specifically inhibiting complement-fixing antibodies derived from other species and the inhibiting property seems to be quantitatively related to the agglutinin antibody content of the serum. The blocking antibody of pollinosis appears to possess an identical inhibiting function, the quantitative aspects of which deserve further study.

SUMMARY

1. The quantitative microprecipitin technique of Heidelberger and MacPherson has been employed in the analysis of factors influencing precipitation of ragweed extracts and rabbit antiragweed serum.

2. Refrigeration for forty-eight hours carried the precipitation to completion. There was least avidity of ragweed extract for rabbit antiragweed antibody in the region of considerable antibody excess.

3. Striking differences were noted for two ragweed extracts in the relation between their protein nitrogen content and the total nitrogen precipitated from a single rabbit serum.

4. The precipitinogenic activity of ragweed extracts diminished progressively during storage in the frozen state.

5. A few preliminary experiments described the effect of "blocking" antibody upon this precipitating system. Normal serums were found to possess some inhibitory activity but much less than that observed with sera from treated ragweed subjects. The degree of inhibition by normal serum was unaffected by altering the conditions of precipitation in a manner which increased the effect of serum from treated ragweed subjects.

6. A given amount of serum containing "blocking" antibody appeared to "neutralize" a fixed amount of antigen independently of the antigen-antibody ratio of the precipitating mixture.

7. The significance of these observations is discussed.

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QUANTITATIVE IMMUNOLOGIC STUDIES WITH ALLERGENS* † ‡

II. Deterioration of Ragweed Pollen Extracts as Measured by Precipitation, Neutralization, and Protein Nitrogen Analysis

The Prevention of Deterioration by Glycerin

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IT IS a well-established fact that various properties of aqueous pollen extracts deteriorate with age and by heating. Clock[1] first reported deterioration of the complement fixation properties of pollen extract at 5° C. in seven weeks. He further showed that storing the extracts in 66⅔ per cent glycerin prevented this phenomenon and suggested that the action of glycerin was in preventing molecular changes due to its hygroscopic features.[2] Paton[3] considered the instability of pollen extracts to be due to the presence of enzymes. Later, Armstrong and Harrison[4, 5] showed that heating low ragweed pollen extract destroyed its capacity to fix complement with rabbit antisera and reported that the complement-fixing properties bore no relation to the actual allergenic factor. Gay[6] found that heating pollen extracts to 56° C. for one hour greatly impaired their activity in skin testing. In 1927, Stier and Hollister,[7] and Alles and Lamson[8] suggested the addition of glycerin to extracting fluids. Stull, Cooke, and Chobot[9] found that ragweed extract, after heating, lost much of its skin test and reagin neutralizing activity.

Stull, Cooke, and Tennant[10] reported that allergenic extracts showed 50 per cent decrease in protein nitrogen after one year's storage at 10° C., although when rediluted gave comparable reactions in skin testing. Arbesman and Eagle[11] reported that heating ragweed extract to 56° C. for 30 and 120 minutes destroyed 75 and 95 per cent of its reagin neutralizing capacity, respectively. They also found that extracts stored in the icebox for two, six, and ten years showed a loss of activity of 84, 95.5, and 98.2 per cent, respectively. The phosphotungstic acid precipitable nitrogen content of the extracts were not changed appreciably. Stull and Sherman[12] reported that 10-month-old low ragweed extract decreased from 40,000 protein nitrogen units per cubic centimeter to 30,000 per cubic centimeter, and that it was much less active in neutralizing human sensitive serum than fresh extract, even when diluted on the basis of 30,000 protein nitrogen units (P.N.U.) per cubic centimeter. Sullivan and Vaughan[13] reported that the skin-reacting factor of pollen extracts did not correspond with the protein nitrogen content and that the presence of glycerin in the extracts protected against deterioration.

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†The study was supported by a grant from the John and Mary R. Markle Foundation.

‡Presented at the Fourth Annual Meeting of the American Academy of Allergy, St. Louis, Mo., Dec. 15 to 17, 1947.

Stull, Cooke, and their co-workers[14] found that heating ragweed extract to 70° C. for one hour reduced the phosphotungstic acid precipitable nitrogen from 0.35 mg. per cubic centimeter to 0.29 (and 0.26) mg. per cubic centimeter although no further reduction occurred in one year. Further studies (Dale and neutralization tests) indicated that the antigenic properties of ragweed pollen extract had been altered by heating. Heated extract did not liquefy gelatin, whereas, unheated extract did. Stull and his associates[15, 16] separated three fractions from low ragweed pollen and observed a loss of protein nitrogen after incubation of two of the three fractions. Stevens[17] incubated giant ragweed pollen extract from six to nineteen days and found a smaller loss of phosphotungstic acid precipitable nitrogen than of nitrogen precipitated by half saturation and full saturation with Na_2SO_4 and suggested, as did Stull,[16] that split protein products may be precipitated by phosphotungstic acid.

This is a report of a study of the deterioration of low ragweed pollen extract by heating and after storage at various temperatures as measured by precipitation of antiragweed rabbit serum and by neutralization of human sensitive serum in the passive transfer test (Prausnitz-Küstner reaction).[18]

METHODS

Five grams of low ragweed pollen were extracted with 100 c.c. of Coca's fluid (0.5 per cent NaCl, 0.35 per cent $NaHCO_3$, and 0.4 per cent phenol) and passed through a Seitz filter. This sterile extract, after analysis by Kjeldahl for its content of phosphotungstic acid precipitable nitrogen, was divided into two portions. Equal parts of Coca's extracting fluid were added to one portion (plain extract), and equal parts of glycerin were added to the other (glycerinated extract) in a final concentration of 50 per cent glycerin. The plain and glycerinated extracts were then stored (a) at room temperature, (b) in the icebox (6° C.), (c) in the deep freeze (–25° C.), and (d) in the carbon dioxide box (–70° C.). The extracts were studied at intervals up to twelve months for their capacity to precipitate antiragweed rabbit serum and to neutralize skin sensitizing antibodies of human ragweed sensitive serum. Similar studies were made with plain and glycerinated extracts subjected to temperatures of 56° C. for thirty minutes in a water bath. Three separate extracts were used in this study, one prepared on Nov. 10, 1946 (Extract A), one on June 21, 1947 (Extract B), and one on Aug. 15, 1947 (Extract C).

A. *Precipitation of Antiragweed Rabbit Serum.*—The absolute amounts of precipitate resulting from union of low ragweed antigen and antiragweed rabbit serum were measured by the use of a photoelectric colorimeter, a method previously described.[19]

B. *Neutralization of Human Ragweed Sensitive Serum.*—The technique employed was that described by Cooke and his co-workers.[20] Two-tenths cubic centimeters of diluted human ragweed sensitive serum was added to 0.2 c.c. of plain and glycerinated low ragweed extracts in strengths ranging from 10 to 2,000 protein nitrogen units per cubic centimeter. The mixtures were shaken and placed in the icebox for one to two hours. Skin sites were prepared in

recipients, reacting negatively to skin tests with low ragweed extract, with 0.1 c.c. of the antigen human serum mixtures. These sites were tested twenty-four hours later with 0.025 c.c. of plain, refrigerated low ragweed extract, containing 1,000 protein nitrogen units per c.c.,[21] of the same lot number as used in the neutralization studies. Reactions at the skin sites were interpreted in twenty to thirty minutes after testing as 4 plus, 3 plus, 2 plus, 1 plus, or negative. The neutralization studies were performed on the same recipients at intervals up to twelve months. Portions of the same human skin sensitizing serum, in a dilution of 1:10, were used in all experiments. This serum had been shown to be positive in passive transfer in several recipients in dilutions ranging from 1:3,000 to 1:5,000.

C. *Standardization of Extracts.*—All extracts were standardized on the basis of their phosphotungstic acid precipitable nitrogen, as described by Cooke and Stull.[21] Analyses were performed in duplicate or triplicate. Dilutions of each extract were then made in protein nitrogen units per cubic centimeter for the precipitation and neutralization studies.

RESULTS

A. *Storage at Room Temperature.*—

1. *Precipitation:* Plain Extract A, prepared Nov. 10, 1946, stored at room temperature, showed almost complete loss of capacity to precipitate antiragweed rabbit serum in twelve months. There was marked loss even after four months. The corresponding glycerinated extract retained essentially all of its activity after four months, although there was noticeable deterioration after one year, as shown in Fig. 1.

2. *Neutralization:* Plain Extract A was first studied in neutralization after it had been stored for 6 months. At first, attempts were made to demonstrate an end point by simple passive transfer methods with the fresh extract, but sharp end points could not be demonstrated within the range desired, so that such attempts were abandoned and neutralization studies were adopted when this extract was six months of age. After six and twelve months of storage at room temperature, the plain extract failed to neutralize even in the concentration of 2,000 P.N.U. per cubic centimeters, whereas, the glycerinated extract neutralized at 400 to 500 P.N.U. per cubic centimeters at six and twelve months (Table I).

B. *Storage in Icebox (6° C.).*—

1. *Precipitation:* Plain Extract A, prepared Nov. 10, 1946, stored at 6° C. showed only slight loss of capacity to precipitate antiragweed rabbit serum in four months but showed some loss of precipitating capacity after twelve months. The corresponding glycerinated extract showed no loss of precipitating capacity after twelve months (Fig. 2). It was noted in this experiment, as well as others, that glycerin exerted an inhibiting effect upon precipitation, most noticeable in concentrations of 25 per cent (5,000 P.N.U. per cubic centimeter of glycerinated extract) and greater (Fig. 7). Further evidence of deterioration of the plain extract is suggested in Fig. 2 by the absence of antigen excess effect depressing precipitation, such as is encountered with fresh extracts. Plain

TABLE I. COMPARISON OF REAGIN NEUTRALIZATION ACTIVITY OF PLAIN AND GLYCERINATED RAGWEED EXTRACTS STORED AT ROOM AND REFRIGERATOR TEMPERATURES

CONCENTRATION OF ANTIGEN (P.N.U. PER C.C.)*	ROOM TEMPERATURE				REFRIGERATOR TEMPERATURE			
	PLAIN		GLYCERINATED		PLAIN		GLYCERINATED	
	6 MONTH	12 MONTH	6 MONTH	12 MONTH	6 MONTH	12 MONTH	6 MONTH	12 MONTH
10							+++	+++
25							++	++
50			+++	+++			+	+
75							0	±
100	++++	++++	++	++	++		0	0
150			+	++			0	0
200			++	+	++	+	0	0
250	++++							
300		++++	+	+	+		0	0
400			±	+	0	+	0	
500	+++	++++	±	±	0		0	
750	+++	++++	0	0	0	0		
1,000	+++	++++	0	0	0	0		
1,500	++	++++				0		
2,000	++	++++				0		
Saline	++++	++++	++++	++++	++++	++++	++++	++++

*Phosphotungstic acid precipitable nitrogen.

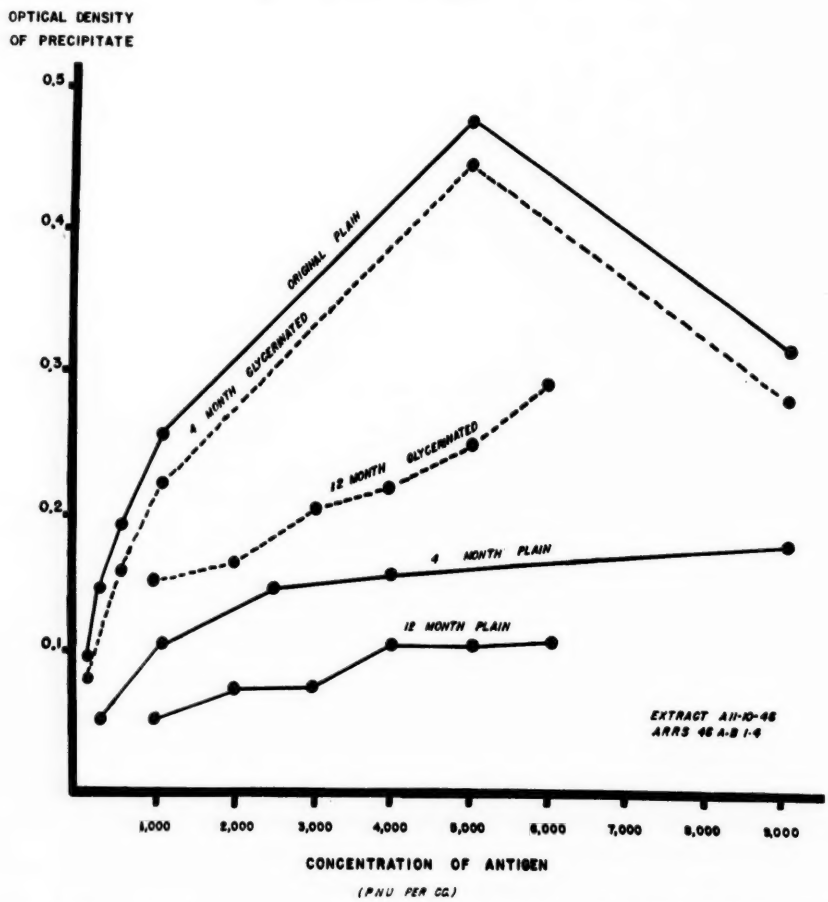

Fig. 1.—Effect of storage at room temperature of plain and glycerinated low ragweed extracts on precipitation of antiragweed rabbit serum.

Extract B, prepared June 21, 1947, showed a significant loss of capacity to precipitate after four months' storage at 6° C., whereas, the corresponding glycerinated extract showed less deterioration (Fig. 3).

2. *Neutralization:* Five hundred to 750 P.N.U. per cubic centimeters of plain Extract A, after twelve months' storage, were required to neutralize human sensitive serum, whereas, neutralization was accomplished at 75 to 100 P.N.U. per cubic centimeter of the corresponding glycerinated extract (Table I). Plain

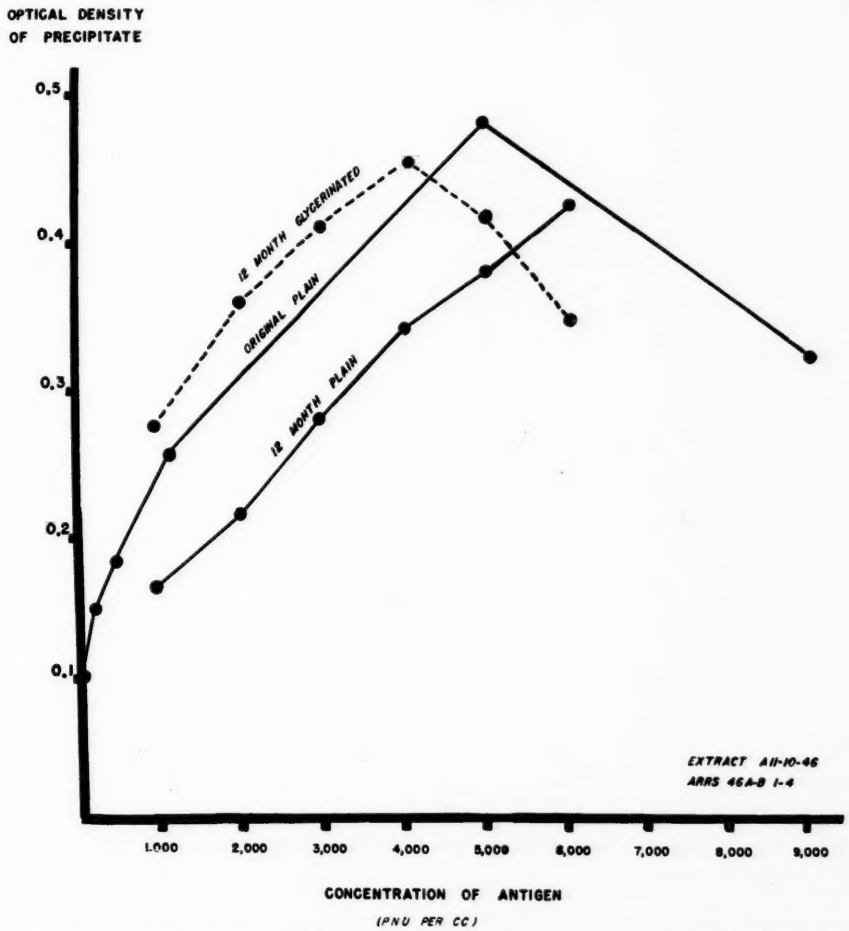

Fig. 2.—Effect of storage at refrigerator temperature of plain and glycerinated low ragweed extracts on the precipitation of antiragweed rabbit serum.

Extract B neutralized at approximately 75 P.N.U. per cubic centimeter when first prepared, although after six months' storage 200 P.N.U. per cubic centimeter of extract were required to induce complete neutralization. The corresponding glycerinated extract, however, showed essentially no loss of neutralizing qualities after six months' storage at 6° C. (Table II).

C. *Storage in Deep Freeze and Carbon Dioxide Boxes.*—

1. *Precipitation:* Plain Extract A, prepared Nov. 10, 1946, showed a marked loss of precipitating capacity after four months' storage in the deep

TABLE II. COMPARISON OF REAGIN NEUTRALIZATION ACTIVITY OF PLAIN AND GLYCERINATED LOW RAGWEED EXTRACTS STORED AT REFRIGERATOR TEMPERATURE

CONCENTRATION OF ANTIGEN (P.N.U. PER C.C.)*	PLAIN		GLYCERINATED	
	ORIGINAL	6 MONTH	ORIGINAL	6 MONTH
10	+++		+++	+++
25	++	++	+++	++
50	+	++	+	+
75	±		±	+
100	0	+	0	0
150	0	+	0	0
200	0	0	0	0
300		0		
400		0		
500		0		
750		0		
Saline	++++	++++	++++	++++

*Phosphotungstic acid precipitable nitrogen.

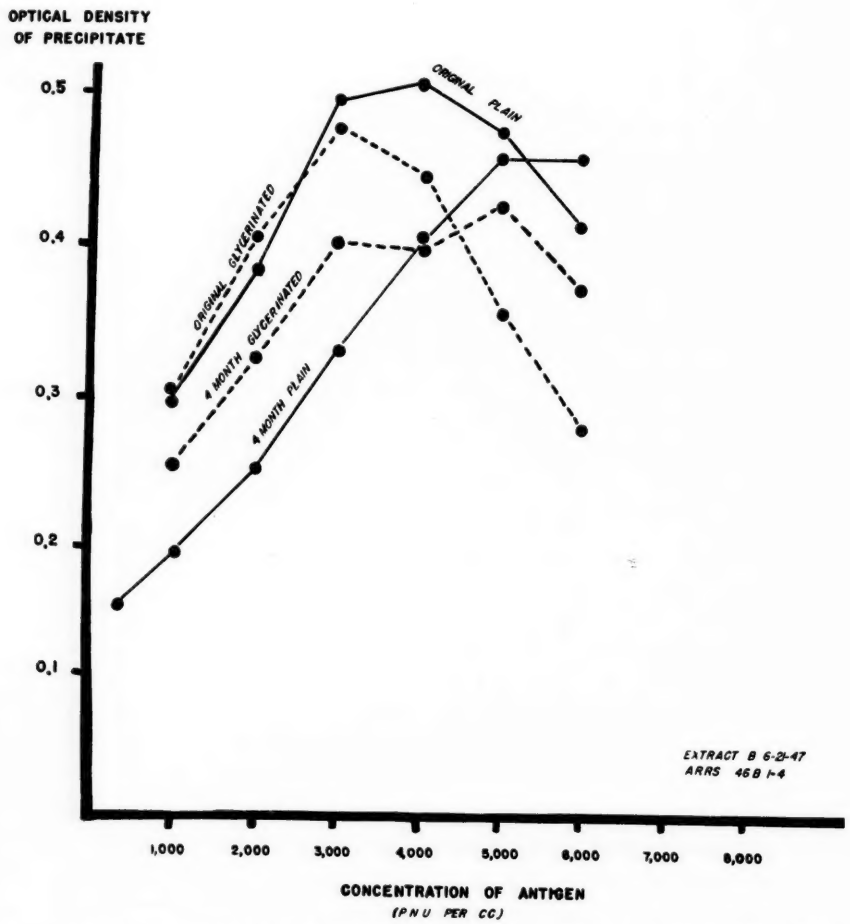

Fig. 3.—Effect of storage at refrigerator temperature of plain and glycerinated low ragweed extracts on the precipitation of antiragweed rabbit serum.

freeze box at –25° C., and in the carbon dioxide box at –70° C. There was slightly more loss in the carbon dioxide box than the deep freeze box but there was no additional loss of activity in either instance after twelve months' storage (Fig. 4). The corresponding glycerinated extracts showed no loss of precipitating capacity, however, after four months or twelve months. The four-month precipitation curves are omitted from the graph shown in Fig. 4 since they overlapped the twelve-month curve.

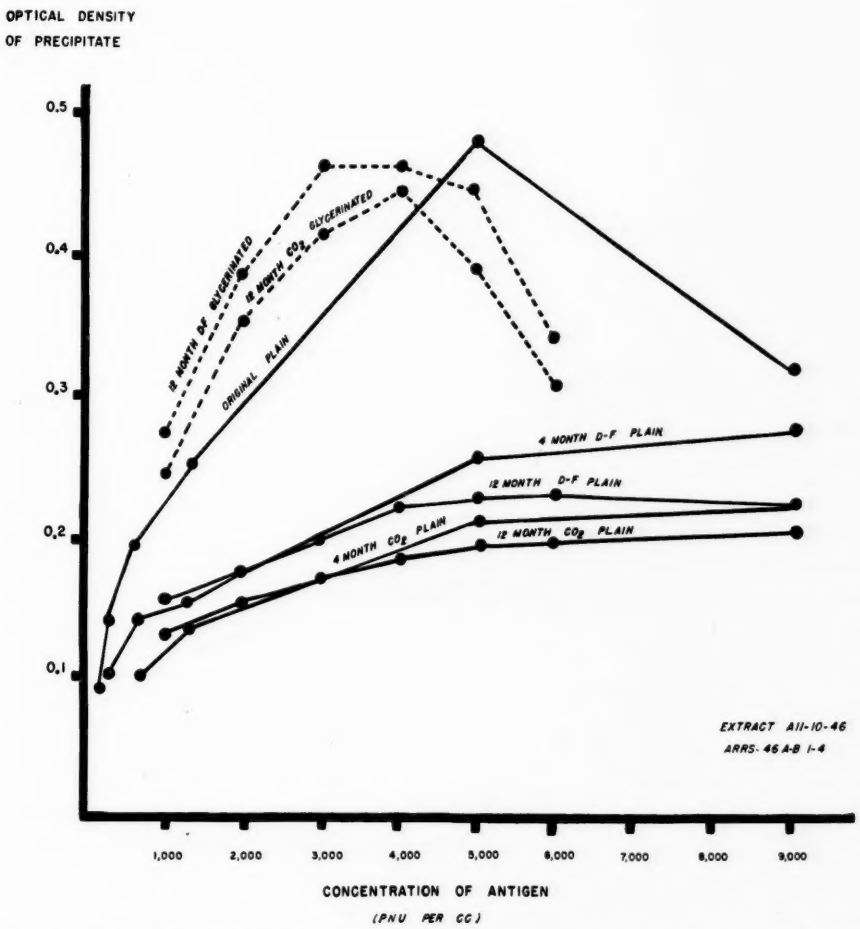

Fig. 4.—Effect of storage at deep freeze and CO_2 box temperatures of plain and glycerinated low ragweed extracts on precipitation of antiragweed rabbit serum.

2. *Neutralization:* Plain Extract A failed to neutralize human sensitive serum even in strengths of 2,000 P.N.U. per cubic centimeter upon analysis after six and twelve months' storage in the deep freeze and carbon dioxide boxes. The corresponding glycerinated extracts, however, neutralized the serum at 75 P.N.U. per cubic centimeter (Table III). Similar loss of activity was noted with Extract B after four months' storage.

D. *Effect of Heat.*—

1. *Precipitation:* Two freshly prepared plain extracts (Extracts B and C), heated in a water bath to 56° C. for thirty minutes, showed marked loss in

TABLE III. COMPARISON OF REAGIN NEUTRALIZATION ACTIVITY OF PLAIN AND GLYCERINATED LOW RAGWEED EXTRACTS STORED AT DEEP FREEZE AND CO_2 BOX TEMPERATURES

CONCENTRATION OF ANTIGEN (P.N.U. PER C.C.)*	DEEP FREEZE				CO_2 BOX				REFRIGERATOR	
	6 MONTH		12 MONTH		6 MONTH		12 MONTH		12 MONTH	
	P	G	P	G	P	G	P	G	P	G
10	++++	+++		+++	++++	++++		++++		++++
25		++		+		++		++		++
50	++++	+		±	++++	+		+		+
75		0		0		±		0		0
100	++++	0		0	+++	0		0		0
150		0		0		0		0		0
200		0		0		0		0	+	0
250	+++		++++		++		++			
400				0				0	+	0
500	+++		++++		++		++			
750	+++				++				0	0
1,000	++		+++		++		++		0	0
1,500					++		.		0	0
2,000	++		+++		++		++		0	
Saline	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

*Phosphotungstic acid precipitable nitrogen.

precipitating properties, whereas, the corresponding glycerinated extracts were not materially affected by heat (Fig. 5). Extract A, after six months' storage in the icebox and then heated, showed similar results.

2. *Neutralization:* Freshly prepared plain Extract B, after heating to 56° C. for thirty minutes, neutralized human sensitive serum at approximately 1,500 P.N.U. per cubic centimeters, whereas, the corresponding glycerinated extract neutralized at approximately 75 P.N.U. per cubic centimeters, the same zone of neutralization observed with the unheated fresh extract (Table IV). This same phenomenon was noted with freshly prepared Extract C, although to a lesser degree. Six-month-old refrigerated Extract A responded similarly to heat in that the heated, glycerinated extract neutralized at approximately 150 P.N.U. per cubic centimeters, whereas, approximately 1,500 P.N.U. per cubic centimeters of unheated plain extract were required to neutralize the serum.

E. *Effect of Glycerin on Precipitation.*—Precipitation studies were performed with varying concentrations of glycerin, i.e., 50, 25, 12.5, 6.25, 3.12, and 1.56 per cent, using a constant amount of antigen (4,000 P.N.U. per cubic centimeter) and antiragweed rabbit serum. Precipitation was inhibited in the presence of the higher concentrations of glycerin up to a maximum inhibition of 18 per cent with 50 per cent glycerin (Fig. 6). This accounts for the depression of the curves of the glycerinated extract at antigen concentrations of 3,000 P.N.U. per cubic centimeter and greater shown in the various figures presented.

F. *Protein Nitrogen Analyses.*—The phosphotungstic acid precipitable nitrogen of all three Extracts (A, B, and C), both plain and glycerinated, decreased after storage at the four different temperatures. The deterioration varied between 30 and 50 per cent. The three extracts did not act alike in this regard. Extract A showed no deterioration after four months' storage at the four different temperatures but approximately 30 per cent after twelve months. There was a decrease of nitrogen after four and one-half months' storage of

TABLE IV. EFFECT OF HEATING PLAIN AND GLYCERINATED LOW RAGWEED EXTRACTS ON REAGIN NEUTRALIZATION

CONCENTRATION OF ANTIGEN (P.N.U. PER C.C.)*	EXTRACT B 6-21-47				EXTRACT C 8-15-47			
	REFRIGERATED		HEATED		REFRIGERATED		HEATED	
	PLAIN	GLYCERINATED	PLAIN	GLYCERINATED	PLAIN	GLYCERINATED	PLAIN	GLYCERINATED
10	+++	+++		+++	++	++		+++
25	++	+++		+	+	++		++
50	+	+		±	+	+		+
75	±	±		±	±	0		+
100	0	0	+++	0	0	0	++	+
150	0	0		0	0	0		0
200	0	0		0	0	0		0
250							+	
500			++				±	
750							0	
1,000			+				0	
1,500			±				0	
2,000			0				0	
Saline	++++	++++	++++	++++	++++	++++	++++	++++

*Phosphotungstic acid precipitable nitrogen.

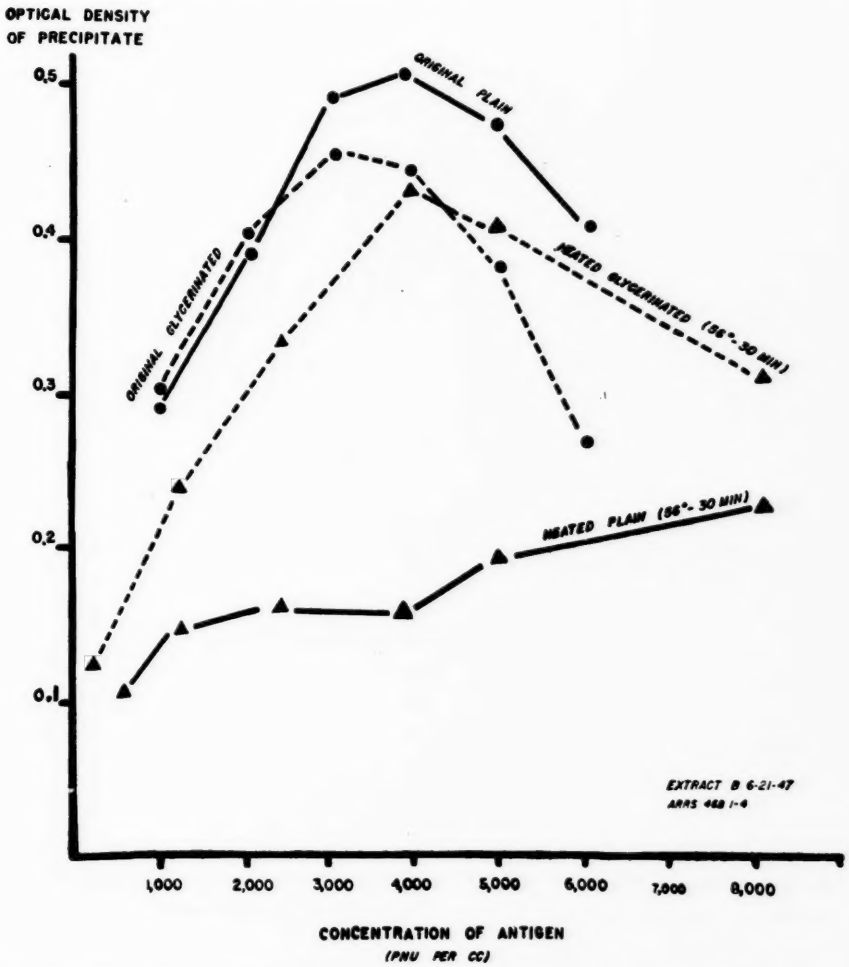

Fig. 5.—Effect of neating plain and glycerinated ragweed extracts on the precipitation of antiragweed rabbit serum.

Extract B and after two and one-half months' storage of Extract C. In general, the values of the glycerinated extracts were less than the corresponding plain extracts, suggesting that glycerin inhibited the precipitation of nitrogen by phosphotungstic acid. There was little or no decrease of nitrogen of the plain

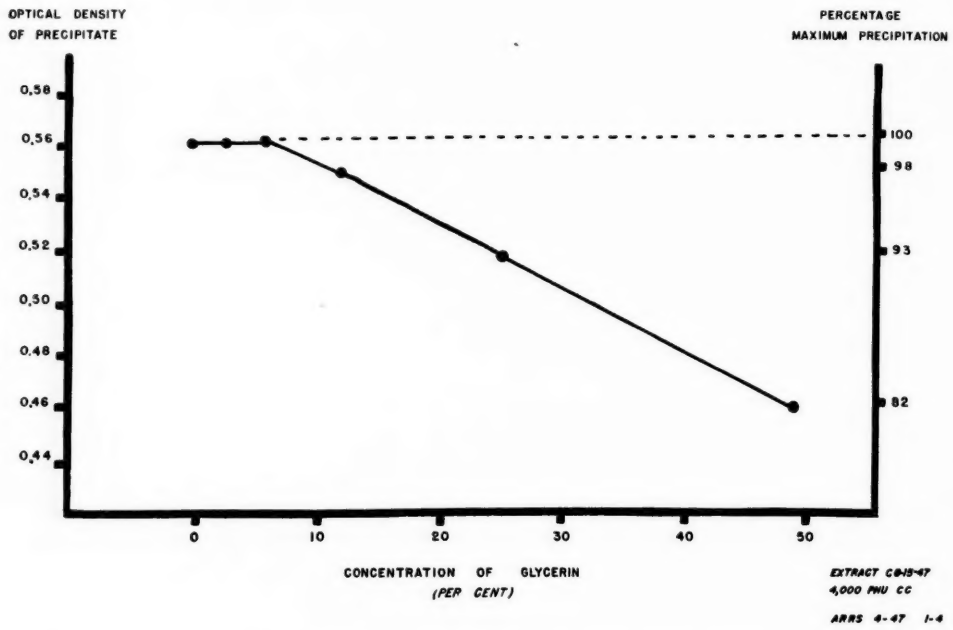

Fig. 6.—Effect of glycerin on precipitation of antiragweed rabbit serum.

TABLE V. PROTEIN NITROGEN ANALYSES* OF EXTRACTS

EXTRACT	DATE	AGE OF EXTRACT	STORAGE CONDITIONS: ROOM P	ROOM G	6° C. P	6° C. G	−25° C. P	−25° C. G	−70° C. P	−70° C. G	HEATED 56° C. P	HEATED 56° C. G
A	11/10/46	Fresh	.10		.10		.10		.10		.10	
	3/19/47	4 mo.	.13	.11	.12	.10	.12	.10	.11	.11	.11	.09
	11/ 3/47	12 mo.	.07	.08	.07	.05	.08	.06	.07	.06		
B	6/21/47	Fresh			.08		.08		.08		.08	
	6/25/47	4 days			.08	.08	.06	.05	.08	.08	.07	.04
	8/19/47	2 mo.					.06	.04				
	11/ 5/47	4½ mo.			.04	.03	.06	.04	.05	.03		
C	8/15/47	Fresh			.10		.10		.10		.10	
	8/19/47	4 days			.09	.07	.08	.07			.09	.06
	11/ 5/47	2½ mo.			.06	.05	.07	.03				

*Phosphotungstic Acid Precipitable Nitrogen per c.c.
P – Plain Extract.
G – Glycerinated Extract.

extracts after heating to 56° C. for thirty minutes although two of the three glycerinated extracts (Extracts B and C) showed consistently less nitrogen after heating (Table V).

DISCUSSION

The results of this study indicate that the capacity of low ragweed extracts to precipitate antiragweed rabbit serum and to neutralize skin sensitizing anti-

bodies of human ragweed sensitive serum is impaired by heating to 56° C. for one hour and by storage at room temperature, 6° C., –25° C., and –70° C. The plain unglycerinated extract deteriorated after storage at the four temperatures although there was less loss of activity in the icebox at 6° centigrade. Glycerin, in a concentration of 50 per cent in the extracts, in general, protected against such deterioration. Even glycerinated extracts deteriorated partially after storage at room temperature for one year.

It was interesting that extracts stored at –25° C. and at –70° C. showed marked loss of activity by the methods used in the study. Precipitation occurred in these extracts after freezing but the precipitates, when separated from the remainder of the extract and redissolved, showed essentially no activity by precipitation of antiragweed rabbit serum or by neutralization of human ragweed sensitive serum.

There was a definite correlation in the results of the deterioration studies as measured by the two different methods, i.e., precipitation of antiragweed rabbit serum and neutralization of human ragweed sensitive serum in passive

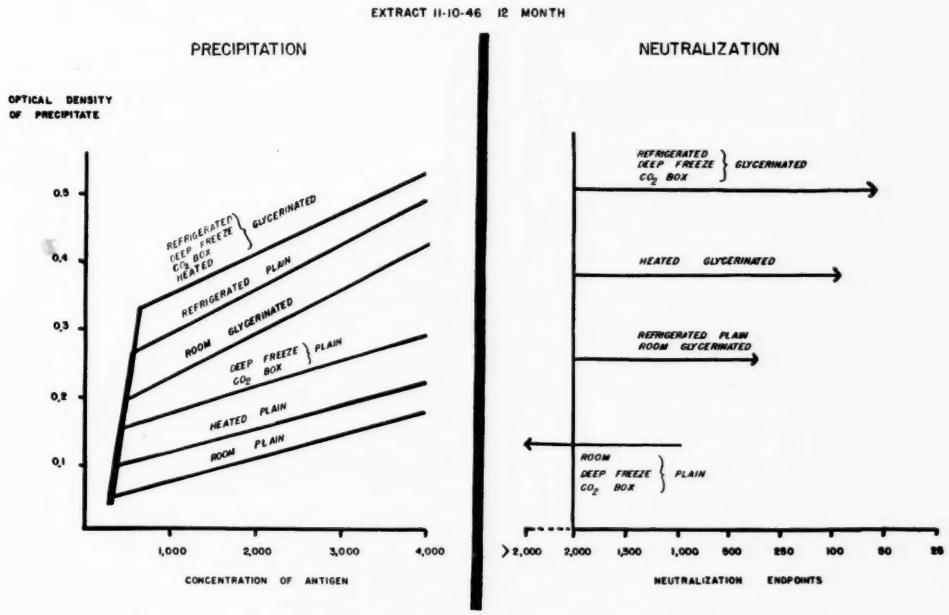

Fig. 7.—Summary of immunologic studies with low ragweed extracts.

transfer. A schematic summary of the results of the effect of heat and the various storage temperatures on deterioration of ragweed pollen extracts indicates this correlation (Fig. 7).

Clinical use of glycerinated extracts has revealed that 50 per cent glycerin irritates human skin inducing "false positive" skin tests and that it is very painful on subcutaneous injection. Even 25 per cent glycerin is painful on injection. In general, extracts containing 5 to 12 per cent glycerin are well tolerated by patients in skin testing and subcutaneous injection therapy. Therefore, 1:4 to 1:10 dilutions of concentrated extracts containing 50 per cent

glycerin are suitable for clinical use. It is possible that a concentration of less than 50 per cent glycerin would prevent deterioration of pollen extracts. Further studies in this regard are being conducted in this laboratory.

There was, in general, loss of phosphotungstic acid precipitable nitrogen in all stored extracts although this loss was not consistent in the three extracts with the same degree of aging. The protein nitrogen content of the plain extracts was not affected materially by heating, although the values were much less after addition of 50 per cent glycerin and heating. It is possible that glycerin inhibited precipitation of the extracts with phosphotungstic acid.

The protein nitrogen of the three extracts studied, as determined by precipitation with phosphotunstic acid, deteriorated 30 to 50 per cent in a year or less. This approximates the findings of Stull, Cooke, and Tennant.[10] There was no correlation, however, between the decrease in nitrogen content and loss of activity of the ragweed extracts by the methods used in this study. The glycerinated extracts, in most instances, showed as much or more loss of nitrogen on storage than the plain unglycerinated extracts, even though glycerinated extracts showed no or much less deterioration as measured by precipitation of antiragweed rabbit serum and neutralization of human ragweed sensitive serum.

SUMMARY

1. Plain unglycerinated low ragweed extracts, upon heating to 56° C. for thirty minutes and upon storage at room temperature, 6° C., –25° C., and –70° C. for periods up to one year, showed loss of activity as measured by their ability to precipitate antiragweed rabbit serum and to neutralize skin sensitizing antibodies of human ragweed sensitive serum.

2. Low ragweed extracts containing 50 per cent glycerin showed no loss or less loss of activity than their corresponding plain extracts after heating or storage at the four different temperatures.

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HISTAMINE ANTAGONISTS*†

A Comparative Study of Their Effect on Histamine and Allergic Skin Wheals

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THE appearance of many new antihistaminic drugs has made it desirable to compare their relative effects by a simple objective method. Friedlaender and Feinberg[1] have reported that Pyribenzamine and Neo-Antergan were probably more potent than Benadryl and Antergan in effecting the whealing mechanism in man. Mayer and his associates[2, 3] found Pyribenzamine to be the most promising of a series of antihistaminic agents (929F, 1571F, 2325RP, and 2339 [Antergan]). These investigations were undertaken with animals, as have been most other comparative studies to date. Friedlaender and associates,[4] using the amount of histamine required to produce a fatal reaction in animals protected by a standard amount of drug (3.0 mg. per kilogram), found Neo-Antergan to be the most potent, with Pyribenzamine next, and Antergan and Benadryl least effective. However, in antianaphylactic studies, these drugs did not exhibit these wide differences, and the four drugs were shown to be equally effective against a single lethal dose of histamine.[5] It was felt that the larger amounts of histamine were necessary to bring out the differences, and that in anaphylaxis, the liberation of histamine is on a much smaller scale. Consequently, the drugs were apparently equally effective in anaphylactic experiments.

Because of these conflicting reports, and the well-recognized dangers of transferring the results of animal experiments to humans, we feel it worth while to report the results of our comparative studies of the antihistaminic and antiallergic properties of several of the newer antihistaminic drugs in humans. Friedlaender and Feinberg[6] have demonstrated the ability of Benadryl and Pyribenzamine, when applied to the skin ten minutes before histamine or other wheal producing agents, to inhibit or diminish the wheal ordinarily produced. Using this phenomenon, we have compared the following antihistaminic drugs‡: Pyribenzamine, Neohetramine, Neo-Antergan, Antistine, Thephorin (Nu 1504), Benadryl, and Histadyl (Antihistamine 01013).

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†Accepted for the program but not presented at the Fourth Annual Meeting of the American Academy of Allergy, St. Louis, Mo., Dec. 15 to 17, 1947.

‡Pyribenzamine (Ciba Pharmaceutical Products, Inc.), Pyridil-N'benzyl-N-dimethylethylenediamine hydrochloride.

Neohetramine (Pyridium Corp. & Wyeth, Incorporated), 2-(N-methoxybenzyl-N-dimethylaminoethyl)-aminopyrimidine monohydrochloride.

Neo-Antergan (Merck & Co., Inc.), Ethylenediamine, N-p-methoxybenzyl-N', N'-dimethyl-N-2-pyridylmaleate.

Antistine (Ciba Pharmaceutical Products Inc.), Phenyl-benzylaminomethyl-imidazoline.

Thephorin (Nu 1504), (Hoffmann-LaRoche, Inc.), 2-methyl-9-phenyl-tetrahydro-1-pridindene acid tartrate.

Benadryl (Parke, Davis & Company), Dimethylaminoethyl benzhydryl ether hydrochloride.

Histadyl (Antihistamine 01013), Lilly, Ely & Company), N-(2-pyridyl)-N-(2-thenyl)-N', N'-dimethyl ethylene diamine hydrochloride.

PROCEDURES

Scratch tests were performed on the flexor surfaces of the forearms of allergic and nonallergic patients. The antihistamine drugs were placed on scratches on the right forearms. As a control, one drop of buffered saline was placed on a scratch on the left forearm comparable to the middle scratch on the right forearm. The solutions were then gently rubbed into the scratches. The relative positions of the drugs were alternated regularly. After ten minutes the arms were washed clean with water and dried. One drop of histamine acid phosphate in buffered saline in a dilution of 1:1,000 (prepared from histamine acid phosphate containing the equivalent of 1 mg. of histamine base) was then placed on all scratch sites. A 1:40 ragweed dilution was used in the antiallergic study. After fifteen minutes the resultant wheals and flares were outlined in ink and traced on transparent paper for a permanent record.

The changes were graded as follows:

0, no change,
1+, less than 50 per cent of diminution of flare and wheal,
2+, more than 50 per cent of diminution of flare and wheal.

RESULTS

A. 1. *Comparison of Antihistaminic Effects of Pyribenzamine and Neohetramine.*—There were twenty patients tested, ten allergic and ten without any evidence of allergy. Pyribenzamine and Neohetramine were used in a 5 per cent dilution in distilled water. Pyribenzamine furnished 2 plus protection in sixteen cases, while Neohetramine furnished 2 plus protection in twelve cases; the latter drug failed five times while there were only three failures for the former. In three instances, Neohetramine offered no protection, while Pyribenzamine furnished 2 plus. Where Pyribenzamine failed, Neohetramine did likewise (Diagrams 1, 2, 3, 4, and Table I).

TABLE I. COMPARISON OF ANTIHISTAMINE EFFECTS OF PYRIBENZAMINE AND NEOHETRAMINE IN 10 NONALLERGIC AND 10 ALLERGIC PATIENTS

TYPE OF PROTECTION	PYRIBENZAMINE						NEOHETRAMINE					
	NON-ALLERGICS		ALLERGICS		TOTAL		NON-ALLERGICS		ALLERGICS		TOTAL	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
0	0	–	2	20	2	10	1	10	4	40	5	25
1+	0	–	2	20	2	10	0	–	3	30	3	15
2+	10	100	6	60	16	80	9	90	3	30	12	60

A. 2. *Comparison of Antihistaminic Effects of Pyribenzamine and Thephorin (Nu 1504).*—This group similarly comprised ten allergic and ten nonallergic patients. Histamine was used in the same dilution but the two antihistaminic drugs were in a 2.5 per cent dilution. In this series, a 2.5 per cent dilution was used because Thephorin is not soluble in a 5 per cent dilution. Pyribenzamine furnished 2 plus protection in all twenty cases, while Thephorin did so in only one-half the patients. In eight instances, Pyribenzamine afforded better than 50 per cent protection (Diagrams 5, 6, 7, 8, and Table II).

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE & NEOHETRAMINE

(Non-allergic Patients)

	A.M.	A.R.	O.G.	F.S.	G.H.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Pyribenzamine 5%					
Histamine 1/1000 10 minutes after Neo-Hetramine 5%					

Diagram 1.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE & NEOHETRAMINE

(Non-allergic Patients)

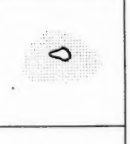
	L.M.	A.V.	O.G.	A.G.	A.S.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Pyribenzamine 5%					
Histamine 1/1000 10 minutes after Neo-Hetramine 5%					

Diagram 2.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE & NEOHETRAMINE

(Allergic Patients)

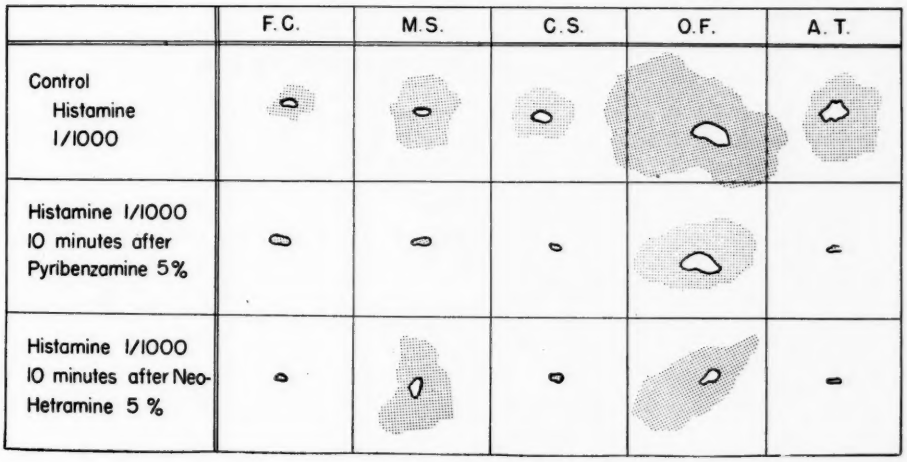


Diagram 3.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE & NEOHETRAMINE

(Allergic Patients)

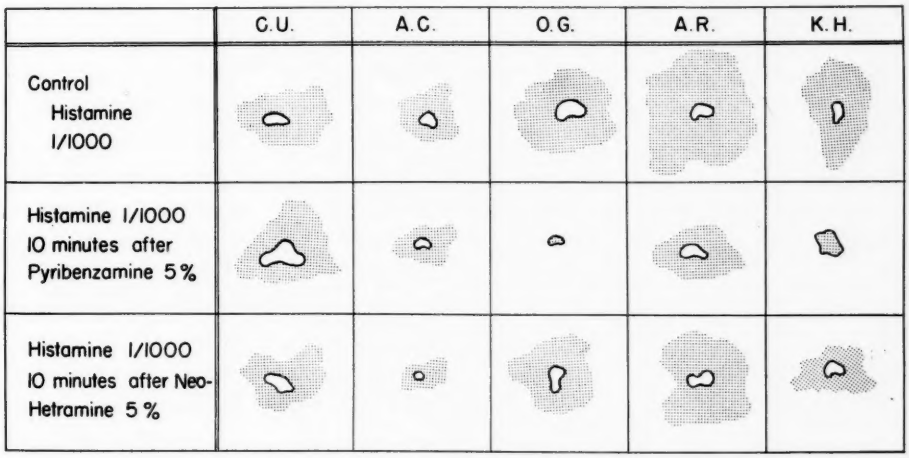


Diagram 4.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF THEPHORIN (Nu 1504) & PYRIBENZAMINE

(Non-allergic Patients)

	A.P. MM5	R.G. WM4	A.N. WM4	A.G. MM5	F.W. MM5
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Thephorin (Nu 1504) 2.5 %					
Histamine 1/1000 10 minutes after Pyribenzamine 2.5%					

Diagram 5.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF THEPHORIN (Nu 1504) & PYRIBENZAMINE

(Non-allergic Patients)

	A.M. WM4	H.G. MM5	R.R. WM4	A.R. WM4	A.G. WM4
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Thephorin (Nu 1504) 2.5 %					
Histamine 1/1000 10 minutes after Pyribenzamine 2.5%					

Diagram 6.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF THEPHORIN (Nu 1504) & PYRIBENZAMINE

(Allergic Patients)

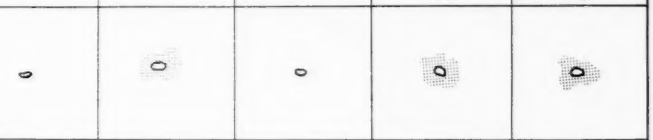
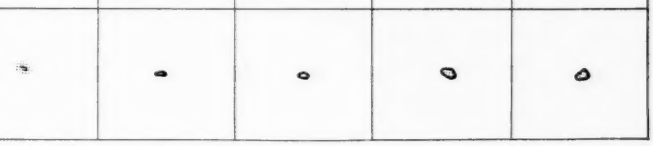
	H.A.	A.G.	S.C.	E.N.	F.C.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Thephorin (Nu 1504) 2.5 %					
Histamine 1/1000 10 minutes after Pyribenzamine 2.5%					

Diagram 7.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF THEPHORIN (Nu 1504) & PYRIBENZAMINE

(Allergic Patients)

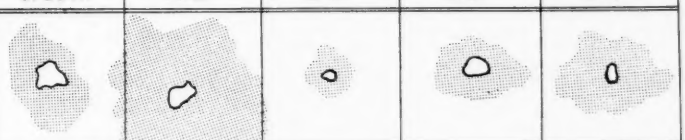
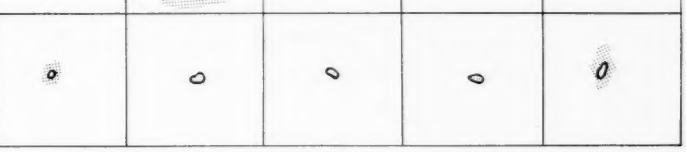
	G. De R.	A.B.	E.D.	A.D.	M.S.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Thephorin (Nu 1504) 2.5 %					
Histamine 1/1000 10 minutes after Pyribenzamine 2.5%					

Diagram 8.

TABLE II. COMPARISON OF ANTIHISTAMINE EFFECTS OF PYRIBENZAMINE AND THEPHORIN (NU 1504) IN 10 NONALLERGIC AND 10 ALLERGIC PATIENTS

TYPE OF PROTECTION	PYRIBENZAMINE						THEPHORIN (NU 1504)					
	NON-ALLERGICS		ALLERGICS		TOTAL		NON-ALLERGICS		ALLERGICS		TOTAL	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
0	0	–	0	–	0	–	3	30	0	–	3	15
1+	0	–	0	–	0	–	4	40	3	30	7	35
2+	10	100	10	100	20	100	3	30	7	70	10	50

A. 3. *Comparison of Antihistaminic Effects of Antistine, Neo-Antergan, and Pyribenzamine.*—This group was similarly constituted as the others. Histamine in 1:1,000 dilution and the antihistaminic drugs in 2.5 per cent dilutions were used, as Antistine is not soluble in 5 per cent dilution. The results were remarkably similar. Neo-Antergan provided 2 plus protection in all twenty patients, while Antistine and Pyribenzamine were almost completely successful in nineteen cases, and each was partially successful in one. In one patient, a striking reactor to histamine, all three drugs furnished good protection objectively, but the patient stated that he felt no itching in the Pyribenzamine scratch but complained of itching over the Antistine and Neo-Antergan as well as the histamine control areas (Diagrams 9, 10, 11, 12, and Table III).

A. 4. *Comparison of Antihistaminic Effects of Antihistamine 01013 (Histadyl), Benadryl, and Pyribenzamine.*—A group of twenty patients, constituted as before, was used. The drugs were used in a 5 per cent dilution and the control sites were treated with 1:1,000 histamine as in the other series. Pyribenzamine and Antihistamine 01013 (Histadyl) furnished 2 plus protection in nineteen and twenty cases, respectively, while Benadryl did so in only seventeen instances. In one instance, Histadyl failed to prevent itching, while the other two drugs did (Diagrams 13, 14, 15, 16, and Table IV).

B. 1. *Comparison of Antiallergic Effects of Pyribenzamine, Thephorin (Nu 1504), Antistine, Neohetramine, Neo-Antergan,* and *Antihistamine 01013 (Histadyl).*—A 1:40 dilution of mixed ragweed was used in a group of twenty patients, most of whom were mild reactors to intradermal tests, but there were a few with moderate reactions. The drugs were used in a 2.5 per cent dilution. The relative positions of the drugs were alternated regularly. Instead of five control sites on the left arm, one scratch was made on the site comparable to the middle scratch on the right arm. Neo-Antergan furnished better than 50 per cent protection in fifteen cases; Pyribenzamine in fourteen; Antistine in thirteen; Neohetramine in ten, and Thephorin in ten. In ten patients Histadyl (01013) was also tried, and in seven protection was of the 2 plus type.

In six patients who were mild reactors, all drugs gave complete protection. In some, where the control reaction was more severe, all drugs failed to furnish 2 plus protection, but they equally protected in a 1 plus fashion (Diagrams 17, 18, 19, 20, 21, and Table V).

B. 2. *Comparison of Antiallergic Effects of Benadryl, Pyribenzamine, and Neohetramine.*—There were ten ragweed-sensitive patients in this series. Pyribenzamine furnished 2 plus protection in six, 1 plus in three, and failed only

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE, ANTISTINE & NEOANTERGAN

(Non-allergic Patients)

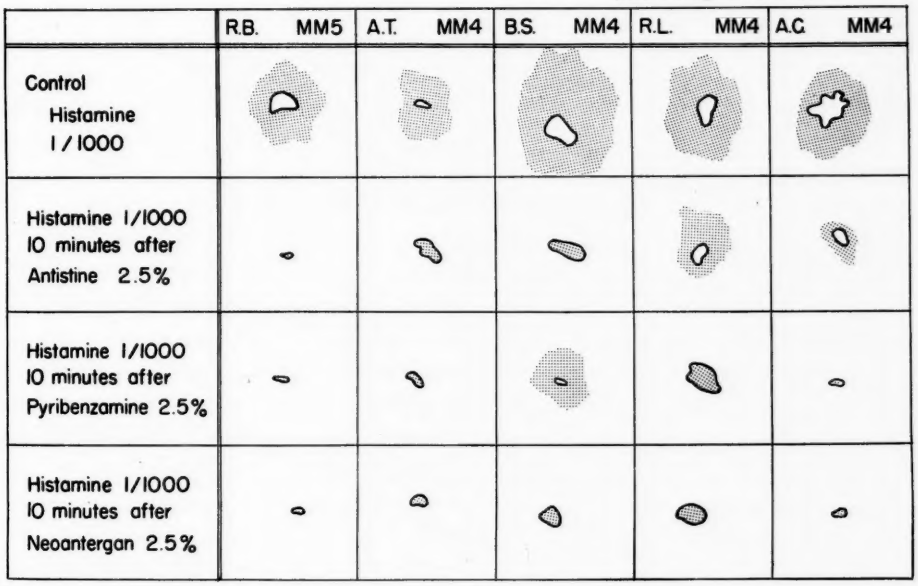


Diagram 9.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE, ANTISTINE & NEOANTERGAN

(Non-allergic Patients)

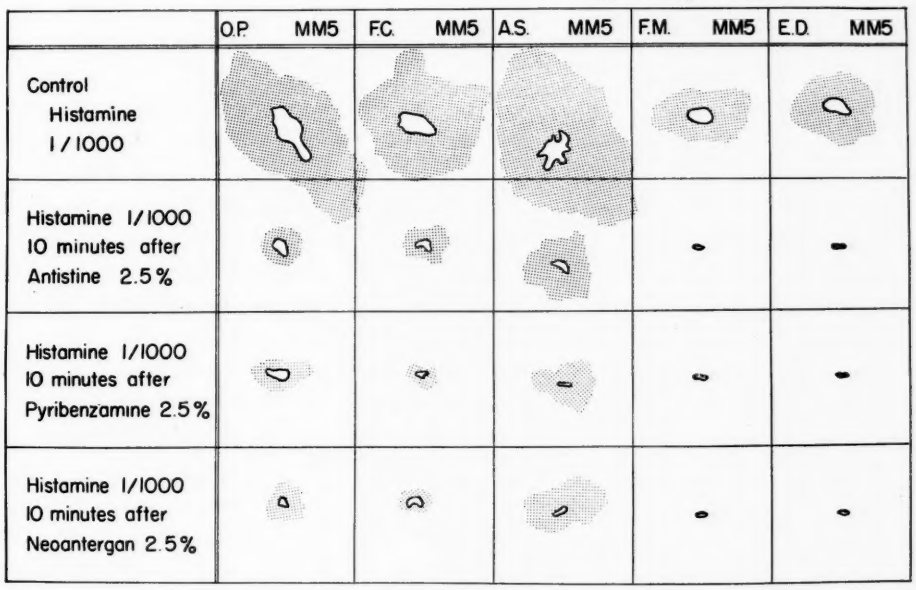


Diagram 10.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE, ANTISTINE & NEOANTERGAN

(Allergic Patients)

	A.M.	T.R.	O.R.	A.H.	B.C.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Antistine 2.5%					
Histamine 1/1000 10 minutes after Pyribenzamine 2.5%					
Histamine 1/1000 10 minutes after Neoantergan 2.5%					

Diagram 11.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE, ANTISTINE & NEOANTERGAN

(Allergic Patients)

	P.C.	A.T.	A.C.	O.B.	A.L.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Antistine 2.5%					
Histamine 1/1000 10 minutes after Pyribenzamine 2.5%					
Histamine 1/1000 10 minutes after Neoantergan 2.5%					

Diagram 12.

TABLE III. COMPARISON OF ANTIHISTAMINE EFFECTS OF ANTISTINE, NEO-ANTERGAN, AND PYRIBENZAMINE IN 10 NONALLERGIC AND 10 ALLERGIC PATIENTS

TYPE OF PROTECTION	ANTISTINE						NEO-ANTERGAN						PYRIBENZAMINE					
	NON-ALLERGICS		ALLERGICS		TOTAL		NON-ALLERGICS		ALLERGICS		TOTAL		NON-ALLERGICS		ALLERGICS		TOTAL	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
0	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–
1+	1	10	0	–	1	5	0	–	0	–	0	–	1	10	0	–	1	5
2+	9	90	10	100	19	95	10	100	10	100	20	100	9	90	10	100	19	95

TABLE IV. COMPARISON OF ANTIHISTAMINE EFFECTS OF HISTADYL (01013), BENADRYL, AND PYRIBENZAMINE IN 10 NONALLERGIC AND 10 ALLERGIC PATIENTS

TYPE OF PROTECTION	HISTADYL (01013)						BENADRYL						PYRIBENZAMINE					
	NON-ALLERGICS		ALLERGICS		TOTAL		NON-ALLERGICS		ALLERGICS		TOTAL		NON-ALLERGICS		ALLERGICS		TOTAL	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
0	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–
1+	0	–	0	–	0	–	0	–	3	30	3	15	0	–	1	10	1	5
2+	10	100	10	100	20	100	10	100	7	70	17	85	10	100	9	90	19	95

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE, ANTIHISTAMINE & BENADRYL

(Non-allergic Patients)

	R. P.	A. O'C.	E. V.	R. L.	A. K.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Pyribenzamine 5 %					
Histamine 1/1000 10 minutes after Antihistamine 01013 5%					
Histamine 1/1000 10 minutes after Benadryl 5 %					

Diagram 13.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE, ANTIHISTAMINE & BENADRYL

(Non-allergic Patients)

	O. H.	A. G.	V. P.	V. F.	J. W.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Pyribenzamine 5 %					
Histamine 1/1000 10 minutes after Antihistamine 01013 5 %					
Histamine 1/1000 10 minutes after Benadryl 5 %					

Diagram 14.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE, ANTIHISTAMINE & BENADRYL

(Allergic Patients)

	A.C.	R.G.	P.C.	V.A.	A.McG.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Pyribenzamine 5%					
Histamine 1/1000 10 minutes after Antihistamine 01013 5%					
Histamine 1/1000 10 minutes after Benadryl 5%					

Diagram 15.

COMPARISON OF ANTI-HISTAMINE EFFECTS OF PYRIBENZAMINE, ANTIHISTAMINE & BENADRYL

(Allergic Patients)

	E.H.	V.T.	R.B.	G.B.	V.A.
Control Histamine 1/1000					
Histamine 1/1000 10 minutes after Pyribenzamine 5%					
Histamine 1/1000 10 minutes after Antihistamine 01013 5%					
Histamine 1/1000 10 minutes after Benadryl 5%					

Diagram 16.

COMPARISON OF ANTI-ALLERGIC EFFECTS
OF PYRIBENZAMINE, THEPHORIN, ANTISTINE,
NEOHETRAMINE, NEOANTERGAN & ANTIHISTAMINE

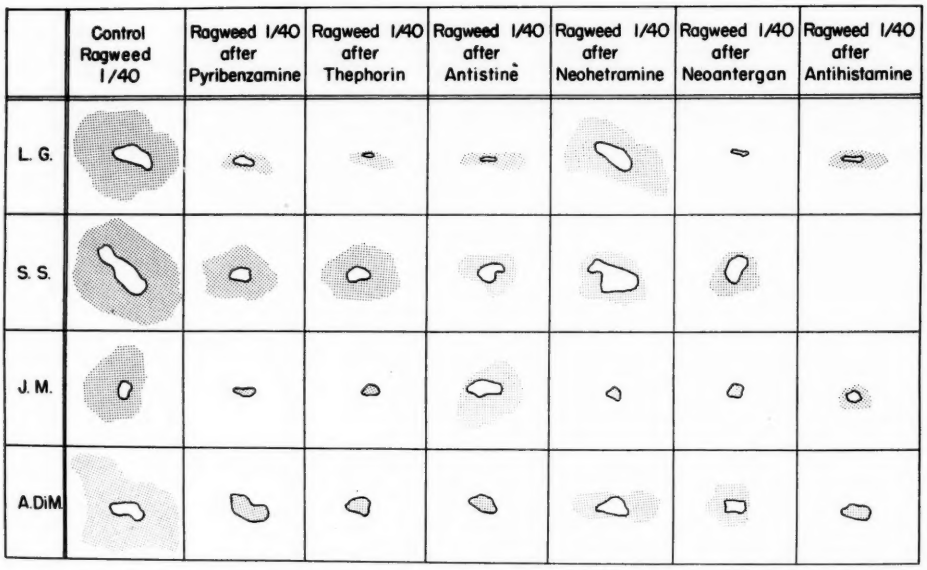


Diagram 17.

COMPARISON OF ANTI-ALLERGIC EFFECTS
OF PYRIBENZAMINE, THEPHORIN, ANTISTINE,
NEOHETRAMINE, NEOANTERGAN & ANTIHISTAMINE

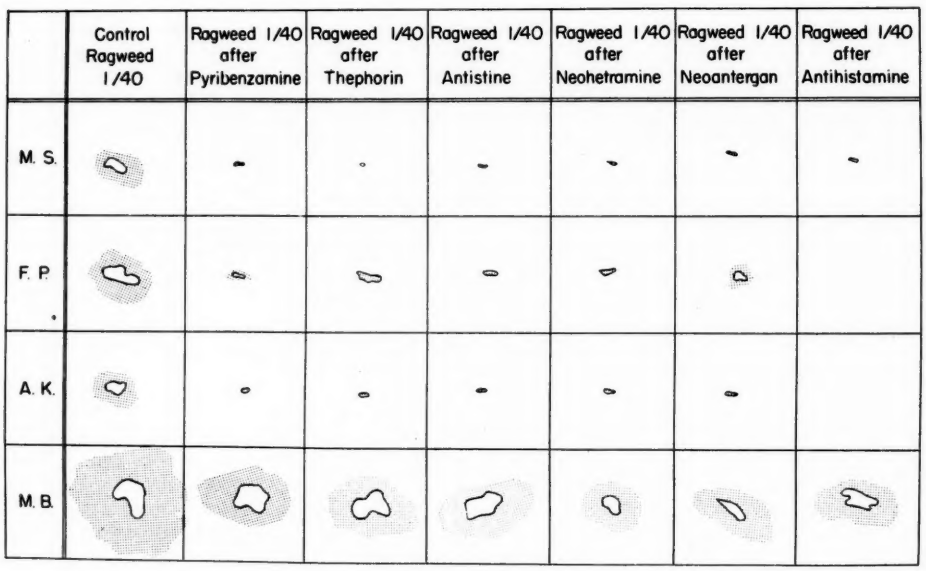


Diagram 18.

COMPARISON OF ANTI-ALLERGIC EFFECTS
OF PYRIBENZAMINE, THEPHORIN, ANTISTINE,
NEOHETRAMINE, NEOANTERGAN & ANTIHISTAMINE

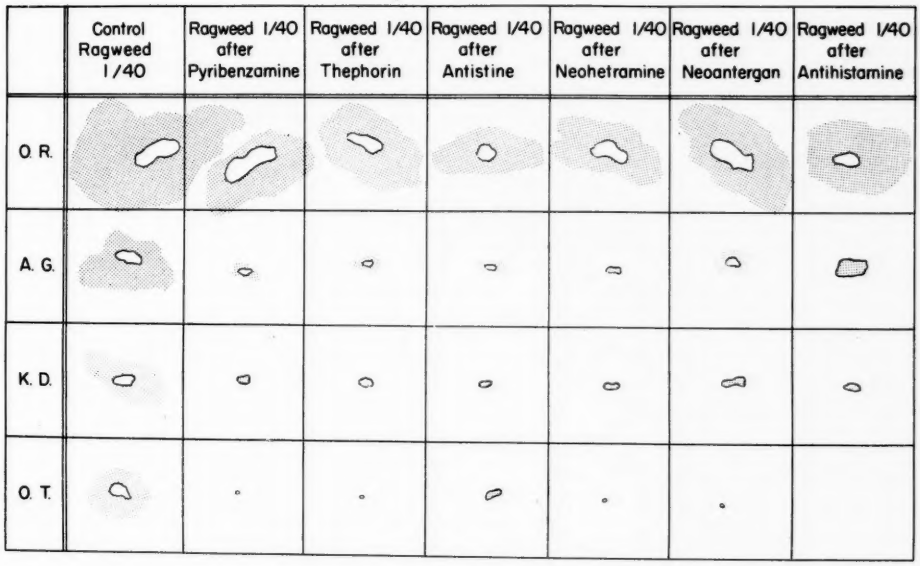


Diagram 19.

COMPARISON OF ANTI-ALLERGIC EFFECTS
OF PYRIBENZAMINE, THEPHORIN, ANTISTINE,
NEOHETRAMINE, NEOANTERGAN & ANTIHISTAMINE

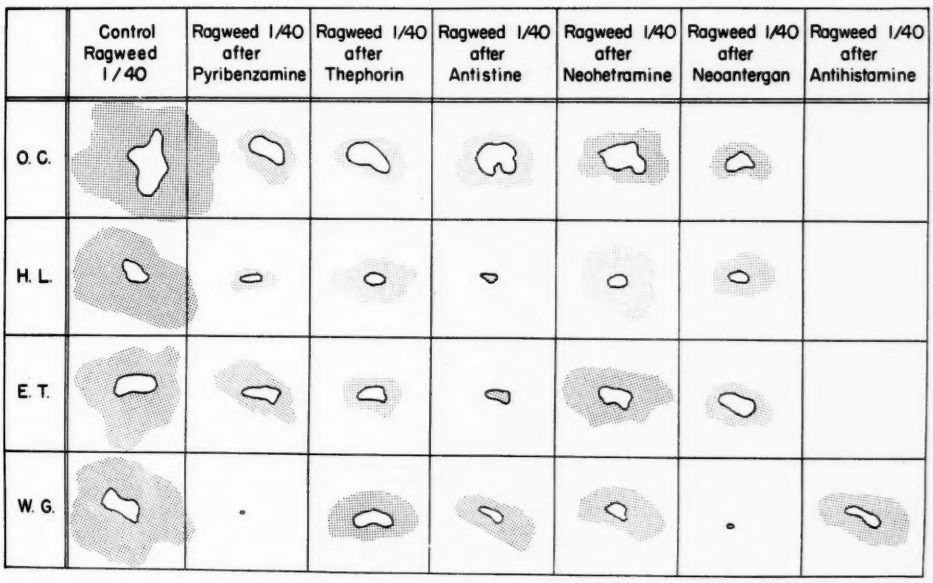


Diagram 20.

TABLE V. COMPARISON OF ANTIALLERGIC EFFECTS OF PYRIBENZAMINE, THEPHORIN, NEOHETRAMINE ANTISTINE, NEO-ANTERGAN, AND HISTADYL (01013) IN 20 RAGWEED SENSITIVE PATIENTS

TYPE OF PROTECTION	PYRIBENZAMINE		THEPHORIN (NU 1504)		ANTISTINE		NEOHETRAMINE		NEO-ANTERGAN		HISTADYL (01013)	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
0	0	–	1	5	2	10	1	5	0	–	0	–
1+	6	30	9	45	5	25	9	45	5	25	3	30
2+	14	70	10	50	13	65	10	50	15	75	7	70

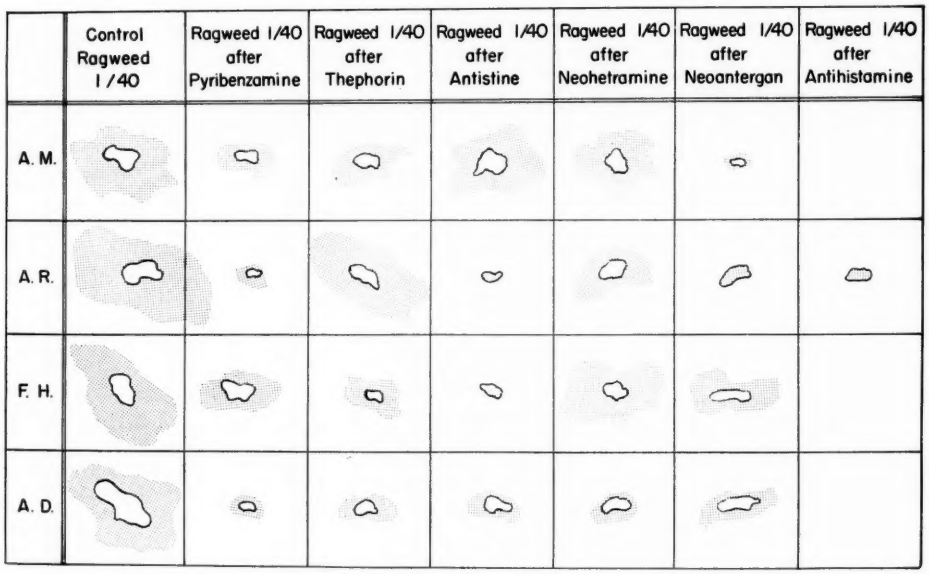


Diagram 21.

once. Neohetramine and Benadryl failed three times, twice furnished 2 plus protection, and five times protected completely against the wheal produced by ragweed 1:40. Here, as in the other groups, all furnished protection against the smaller reactions (Diagrams 22, 23, and Table VI).

TABLE VI. COMPARISON OF ANTIALLERGIC EFFECTS OF BENADRYL, PYRIBENZAMINE, AND NEOHETRAMINE IN 10 RAGWEED SENSITIVE PATIENTS

TYPE OF OF PROTECTION	BENADRYL		PYRIBENZAMINE		NEOHETRAMINE	
	NO.	%	NO.	%	NO.	%
0	3	30	1	10	3	30
1+	2	20	3	30	2	20
2+	5	50	6	60	5	50

COMPARISON OF ANTI-ALLERGIC EFFECTS OF BENADRYL, PYRIBENZAMINE & NEOHETRAMINE

	D. K.	R. T.	G. T.	L. DeL.	A.M.
Control Ragweed 1/40					
Ragweed 1/40 10 minutes after Benadryl					
Ragweed 1/40 10 minutes after Pyribenzamine					
Ragweed 1/40 10 minutes after Neohetramine					

Diagram 22.

COMPARISON OF ANTI-ALLERGIC EFFECTS OF BENADRYL, PYRIBENZAMINE & NEOHETRAMINE

	A. D.	H. L.	R. T.	R. K.	A. R.
Control Ragweed 1/40					
Ragweed 1/40 10 minutes after Benadryl					
Ragweed 1/40 10 minutes after Pyribenzamine					
Ragweed 1/40 10 minutes after Neohetramine					

Diagram 23.

DISCUSSION

Our findings seem to confirm the work of Rose and his associates.[5] Apparently, in human subjects, most of the newer antihistaminic drugs are qualitatively alike and show quantitive differences only when the stimulus is great. In the series using a strong ragweed extract as the stimulus, the drugs may be arranged as follows in order of decreasing effectiveness:

1. Neo-Antergan (75 per cent of twenty cases)
2. Histadyl (01013) (70 per cent of ten cases)
3. Pyribenzamine (66.6 per cent of thirty cases)
4. Antistine (65 per cent of twenty cases)
5. Thephorin (Nu 1504) (50 per cent of twenty cases)
6. Neohetramine (50 per cent of thirty cases)
7. Benadryl (50 per cent of ten cases)

Similarly, the drugs may be listed as to their antihistaminic effects as:

1. Neo-Antergan (100 per cent of twenty cases)
2. Histadyl (01013) (100 per cent of twenty cases)
3. Antistine (95 per cent of twenty cases)
4. Pyribenzamine (92.5 per cent of eighty cases)
5. Benadryl (85 per cent of twenty cases)
6. Neohetramine (60 per cent of twenty cases)
7. Thephorin (Nu 1504) (50 per cent of twenty cases)

It will be noted that generally the drugs are grouped the same in both the antiallergic and antihistaminic experiments. However, they did not furnish the same high percentage of protection in the antiallergic group. This is probably due to two reasons: (1) that liberation of histamine is not the only cause of the allergic wheal, and (2) the intensity of the stimulus in many of the patients was probably greater than that afforded by 1:1,000 dilution of histamine. In the mild reactors to ragweed, all drugs gave complete protection. With stronger stimuli, differences may be brought out, but we feel that these patients are within the range of the sort of stimuli occurring in allergic patients and therefore, of greater value than when unusually strong stimulation is used.

SUMMARY

1. An objective method for comparing the antihistaminic drugs in humans by use of the multiple scratch test is used to compare the antihistaminic properties of Pyribenzamine and Neohetramine; of Pyribenzamine and Thephorin; of Antistine, Neo-Antergan, and Pyribenzamine; and of Histadyl (01013), Benadryl, and Pyribenzamine.

2. By the same technique, the ability of these drugs to alter the wheal produced by ragweed in sensitive patients is compared.

3. The drugs can be arranged into three groups as far as their antihistaminic properties are concerned. Neo-Antergan, Histadyl (01013), Pyribenzamine, and Antistine are more effective in a significant proportion of cases than Benadryl, which is itself more effective than Neohetramine and Thephorin.

4. Neo-Antergan, Histadyl (01013), Pyribenzamine, and Antistine are more effective than Thephorin (Nu 1504), Neohetramine, and Benadryl in altering the allergic wheal in humans.

5. It is felt that the differences between the members of the group are not significant enough in this series to permit adequate rating, if indeed one is better than the other. It is probable that only when the stimulus is much stronger than that occurring naturally that any significant differences can be exhibited.

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EXTRACTS OF MOLDS GROWN ON SYNTHETIC MEDIA

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John W. Argabrite, M.S., M.D., Watertown, S. Dak.

THE importance of airborne mold spores as a cause of allergic disease has been well established by Feinberg,[1] Prince and Morrow,[2] Morrow and Lowe,[3] Bieberdorf and Hampton,[4] as well as others.

Although good results have been obtained with the use of extracts prepared from molds grown on glucose peptone broth as reported by Hampton and Lowe,[5] there is the possibility that the proteins from the peptone broth may interfere in the correct standardization of the extract by the Cooke-Stull method.[6] With this in view, a project was initiated to grow the molds on a protein-free media.

PREPARATION OF EXTRACTS

The eight most common airborne molds as reported by Bieberdorf and Hampton[4] are Hormodendrum, Alternaria, Spondylocladium, Helminthosporium ("Hash"), Fusarium, Aspergillus, Phoma, and Pencillium ("Fapp") were used in this research. Although numerous protein-free formulas were tried, the following was found to be the most successful for all eight molds.

Sucrose	30.00 grams
$NaNO_3$	2.00
K_2HPO_4 Diabasic	1.00
KH_2PO_4 Monobasic	.50
$MgSO_4$	.50
KCl	.50
$FeSO_4$	.01
H_2O Distilled Q.S.A.D.	1,000.00 milliliters

The molds were grown from three to five weeks at room temperature in 1,000 ml. Erlenmyer flasks to which 200 ml. of the media had been added. The media were sterilized for eighteen minutes at 15 pounds pressure.

The pellicles were removed from the flasks, washed once with sterile saline, and ground in a Waring blendor to which approximately 100 ml. of Coca's extracting fluid with phenol was added for each series of eight mold pellicles. The macerated material was placed in a refrigerator with the extracting fluid for extraction for forty-eight hours. It was then centrifuged, the liquid poured off, and filtered through a Seitz filter. The amount of extract obtained in this procedure was approximately 90 milliliters. Extracts, prepared as listed above, were compared with extracts prepared with a peptone-broth extract according to the procedures outlined by Hampton and Lowe.[5] Both the peptone-free and peptone-mold extracts were freshly prepared so that they were comparable in regard to age.

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RESULTS

In standardization of mold extracts prepared by use of peptone-free media we were unable to approximate even within wide margins the amount of protein nitrogen units (P.N.U.) per milliliter as was formerly obtained by the peptone-broth method. The results are presented in Table I. The wide variation in protein nitrogen units per milliliter of the two extracts led to the following studies in order to determine the source of the additional protein nitrogen units per milliliter in the peptone-broth extract. A one hundred ml. sample of each extract was concentrated to fifty ml. under negative pressure. Each extract was precipitated by heating with varied concentrations of ammonium sulfate to remove the protein and the protein-like substances.[7] After all the protein products were removed by heating and saturation with ammonium sulfate, the filtrates were tested for peptone by the Biuret method.[8] The extract prepared from the mold grown on the peptone media gave very positive results for peptone by this method. The extract produced from the mold grown on the synthetic media gave almost negative results for peptone. To the remaining portion of the filtrates, barium carbonate was added. The combination was boiled and the ammonium sulfate removed as barium sulfate by filtration. At this point, a micro-Kjeldahl standardization procedure for determining protein nitrogen units was done on each filtrate. The "peptone"-produced extract filtrate gave a protein nitrogen unit value from 5,000 to 20,000 per milliliter of the original filtrate. The "synthetic"-produced extract filtrate gave nontitratable amount on the Kjeldahl process. Kjeldahl analyses were also performed on each of the culture mediums used after removal of the mold pellicles. The peptone media gave a very high protein nitrogen units per milliliter, and the "synthetic" media, a nontitratable amount. Kjeldahl determinations were done on the washing of the mold pellicles grown on both types of media. The first washing from the peptone-grown pellicle gave a protein nitrogen units of 5,000 per milliliter. The solution from each consecutive washing showed a reduction in protein nitrogen units; the fifth washing being practically negative. The first washing from the "synthetic"-grown pellicle gave practically negative results.

TABLE I. AVERAGE P.N.U.* PER C.C.

	PEPTONE-FREE MEDIA	PEPTONE MEDIA
Alternaria	15,666	72,714
Spondylocladium	8,400	41,800
Phoma	14,000	35,857
Penicillum	19,000	57,142
Hormodendrum	12,000	57,600
Helminthosporium	22,300	52,400
Fusarium	13,500	51,500
Aspergillus	10,000	40,285

*Protein Nitrogen Unit.

As a result of the above findings a plan for clinical investigation of the two extracts were initiated.

In order to compare the chemical findings with a bio-assay, twelve patients were skin-tested with the old extract, new extract, other pollens and inhalants,

and diluting fluid. The skin reaction to 0.02 c.c. of 1,000 protein nitrogen units is recorded as: (a) negative—no reaction; (b) slight—a small wheal less than 1.0 cm. in diameter; (c) moderate—a wheal 1.0 cm. in diameter without pseudopodia; (d) marked—any reaction with pseudopodia; and (e) any reaction that was over and above marked reactions was graded as marked one plus, marked two plus, etc. The results are presented in Table II.

TABLE II. SKIN REACTIONS TO POLYMOLD EXTRACTS

PATIENT	PEPTONE EXTRACT	PEPTONE-FREE EXTRACT
1. A. H.	Fapp—mod*	Mrk
2. I. N.	Hash—sl*	Sl
	Fapp—sl	Mrk
3. T. W.	Hash—mod	Mrk
	Fapp—mod	Mrk
4. E. B.	Hash—mod	Mrk
	Fapp—mod	Mrk
5. W. D.	Hash—mrk*	Mrk XXX*
6. V. J.	Hash—mrk	Mrk
	Fapp—mod	Mrk
7. G. H.	Hash—mod	Mrk
	Fapp—mrk	Mrk
8. B. R.	Hash—mrk	Mrk X*
	Fapp—mrk	Mrk X
9. E. D.	Hash—mod	Mrk
	Fapp—mod	Mrk
10. P. R.	Hash—mrk	Mrk X
	Fapp—mod	Mrk
11. T. F.	Hash—mrk	Mrk X
	Fapp—mod	Mrk
12. M. T.	Hash—mrk	Mrk X
	Fapp—mod	Mrk

*Slight = wheal less than 1 centimeter in diameter.
*Moderate = wheal 1 centimeter in diameter without pseudopods.
*Marked = any wheal with pseudopods.
*Marked X = larger wheal with more prominent pseudopods.
*Marked XXX = larger wheal with very prominent pseudopods.

Three of the twelve patients, A. H., I. N., and T. W., were untreated. The remaining patients have been treated from two weeks to one year. Patient T. W. was found sensitive to molds only, whereas A. H. and I. N. were sensitive to dust also. In only two (E. B. and W. D.) of the twelve patients was there found a pollen sensitivity. From their clinical history, unreactiveness to other allergens, and constitutional reactions to the mold extracts apparently in seven of the cases (V. J., G. H., B. R., E. D., P. R., T. F., and M. T.), molds constitute their principal allergens. In all twelve patients the extract prepared without the use of peptone caused a greater, or at least an equal, skin reaction as compared with the peptone-broth extract when diluted to corresponding strengths. Eighty-nine patients were used as controls. Twenty of these were normal subjects and gave no history of allergy; the rest gave histories consistent with sensitivity to allergens, and these all showed negative skin reactions to either the extract from synthetic media or peptone-broth mold extracts.

Even though the results from chemical assay and skin tests indicate that the peptone-free extract was more potent than the peptone-broth extract of the same protein nitrogen units, it was decided to observe its effects when substituted

in treatment for the peptone-broth extract without reducing the dosage. The results are presented in Table III. All these patients had reactions ranging from local to severe constitutional reactions necessitating emergency treatment. It was then found that by reducing the dosage to the amount listed, these patients were carried along without symptoms for over one month. Also, two patients, J. A. and H. T., who had been receiving "Fapp" 3,000 and 600 P.N.U., respectively, were effectively treated with 1,000 and 80 P.N.U., respectively.

TABLE III. THERAPEUTIC EVALUATION

PATIENT	OLD DOSAGE	REACTION—USING NEW EXTRACT WITHOUT CHANGING DOSAGE	NEW DOSAGE (PNU)
M. T.	Hash 600 Fapp 700	Sore arm (angioneurotic edema of eyelids)	100
T. F.	Hash 1000 Fapp 1500	Local Reaction, Sneezing, Stuffiness, Asthma	150
E. D.	Hash 500 Fapp 600	Sore Arm	200
B. R.	Hash 200 Fapp 300	Sore Arm	25
V. J.	Hash 300 Fapp 400	Sore Arm	150
G. H.	Hash 500 Fapp 500	Sore Arm	250
J. A.	Fapp 3000	New Ext. reduced because of high dosage	1,000
H. T.	Fapp 600	New Ext. reduced because of high dosage	80

In reviewing the old records it was not uncommon to find patients built up to 1,000, 3,000, or 4,000 P.N.U. of both "Hash" and "Fapp." However, with the use of the peptone-free extract we have been unable to go above 1,000 P.N.U. in treatment without getting systemic reactions. In fact it is more of an exception than the rule to find a patient requiring more than 300 P.N.U.

Between April and July, the season that finds mold spores very abundant in the air in the region of San Antonio, 122 patients were tested, grading the skin reactivity as negative, slight, moderate, and marked. Only the moderate and marked reactions were reported as significantly positive skin tests. Twenty seven per cent showed positive skin reactions to molds. Of the total, 22 per cent were sensitive to "Hash," 18 per cent had marked reactions, and 4 per cent had moderate reactions. Twenty per cent were sensitive to "Fapp," 14.3 per cent were marked, and 5.7 per cent were moderate reactions. Eighteen per cent were sensitive to both "Hash" and "Fapp." Of the mold-sensitive patients, 88 per cent had respiratory allergy. It is too early to evaluate the clinical significance of the above findings. The above figures compare quite well with studies of Harris,[9] Chobot, Dundy, and Schaffer[10] on their studies with skin reactions to Alternaria and Aspergillus, and also Feinberg's findings[11] on molds in general. These percentages fall within the range obtained by Zink[12] who found that airborne molds were important clinically in 25.2 per cent of patients tested and treated. These percentages are greater than those of Bieberdorf and Hampton,[4] who found that 12.28 per cent of 1,515 patients revealed marked skin reactions with mold extracts prepared from pellicles grown on peptone media.

DISCUSSION

Although the mold pellicles grown on protein-free media did not make as heavy growth as when peptone was added, possible variation of the above formula might give better results. In preparation of extracts by the old method using glucose peptone broth it was never certain how much of the peptone was included in the mycelium mat and even though it was washed several times, it was thought that it was impossible to remove all the peptone from this mass. How much this peptone raised the amount of protein nitrogen units on assay of the extracts was unknown. It is apparent from the figures presented in Table I and the other chemical analyses, using the same species of fungi, that this extraneous nitrogen has influenced the total protein nitrogen units a great deal.

It has always been felt that this excess nitrogen is innocuous, and Selle[13] in his work was unable to find any histamine or histamine-like substances in the washings or the concentrated extracts employed in skin testing. Hanke and Koessler[14] found a histamine dichloride in the peptone of some of the commercial preparations, which in our opinion adds another skin irritant to mold extracts. Preparation of the mold extracts by the method discussed removed the possibility of histamine from the breakdown products of peptone, thus eliminating false positive skin reactions. It also avoids the useless procedure of hyposensitization to peptone. All the ingredients used in the media were selected because of their high degree of solubility and thus would be readily removed by a single washing of the mold pellicles.

The extracts prepared by peptone-free media eliminated the additional number of washings. It is our opinion that several washings of the mold pellicles prepared by peptone-broth media may eliminate excess peptone in the final extract, but in doing so reduces the antigenic potency of the extract by absorption of some of the mold protein.

It is apparent from the group of controls and the percentage of positive skin reactions that these extracts are no more irritant than extracts made by other procedures. Bierberdorf and Hampton[4] reported in their studies at this laboratory and clinic in 1945 that 12.28 per cent showed significantly positive skin tests (pseudopodia). In comparing their extracts with those used in this study it has been demonstrated that some of their patients now show a more positive skin reactivity using the same amount of protein nitrogen units per milliliter in skin testing. It is possible, by using the peptone extracts, that they were rejecting some mold patients because they did not show sufficiently positive skin tests. Therefore, if we were to include these patients, their figures would compare more closely to those herein reported.

SUMMARY

1. A method is described for preparing mold extracts which eliminates the use of peptone in media.

2. Mold extracts thus prepared are more potent than the former extract of the same protein nitrogen unit value.

3. These mold extracts prepared by the synthetic media were apparently not irritating.

4. Synthetic mold extracts eliminate additional washings of the mold pellicles.

ACKNOWLEDGMENT

We would like to express our appreciation to Arthur A. Wykes, Colonel D. W. Kuhns, M. C., Mr. David Mitzkus, and the rest of the staff of the Fourth Army Area Medical Laboratory for the preparation, standardization, and chemical analysis of the mold extracts. Without their help, this study could not have been made.

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SKIN SENSITIVITY TO STREPTOMYCIN*†

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SINCE January of 1944, when streptomycin was first introduced by Waksman, Bugie, and Schatz,[1] numerous toxic reactions following injection or contact with the drug have been described.[2] It is widely known wherever the drug is used that contact dermatitis frequently follows extensive handling[3, 4]; but few cases of this have been reported and the literature so far contains nothing about the optimum strength of solution to be used for patch testing nor the precautions necessary for prophylaxis, and finally treatment of the contact dermatitis once it has developed.[5, 6] In the tuberculosis hospital of the Veterans Administration Center at Dayton, Ohio, about 750 Gm. of streptomycin are used every month for treatment. As far as patients' reactions to injections of the drug are concerned, there have been none serious enough to have made necessary withdrawal of streptomycin treatment. No cases of contact dermatitis have followed the injection of the drug.

Streptomycin is prepared for injection by dissolving 1 Gm. in 2 ml. of distilled water or 5 Gm. in 10 ml. of distilled water, thereby making a 50 per cent solution of streptomycin for injection. By standing order, the syringes are supposed to be washed by the nurses giving the injections before being sent to Central Supply where they are rewashed, dried, wrapped, and then autoclaved. Approximately 200 syringes per day go through this procedure, the majority of which have been used to administer streptomycin. Previously, it had been recommended that rubber gloves be used when handling these syringes, but this was not definitely ordered and so was rarely done. If skin sensitivity to streptomycin could be developed easily, certainly it would be expected to develop first in the nurses and attendants who handled most of the streptomycin syringes; namely, the personnel working in Central Supply, consisting of two nurses and one female attendant. Their case histories are elaborated in detail below.

CASE REPORTS

Case 1.—F. W., a 36-year-old nurse, reported to the Allergy Clinic on Feb. 10, 1948, complaining of a rash on both hands and arms. She stated that the last time she had had any rash was in 1936 when one developed on both hands and wrists, at which time soap powder was suspected. The eruption healed spontaneously in about ten days. The present rash began about Dec. 15, 1947, at the base of the fourth finger of the left hand under the patient's wedding ring. From there it spread to other fingers, involving first the interdigital spaces. The eyelids were involved at the onset and presented a reddened, swollen, scaling appearance.

This nurse had been in contact with streptomycin almost daily since June 27, 1947. She was also in contact with alcohol, face powder, rouge, lipstick, hand lotion, and shampoo, but no nail polish was used. When the rash first appeared, she stated there were

*From the Allergy Service, Veterans Administration Center.

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small fluid-filled blisters which dried, leaving deep fissures. The patient was sent to a dermatologist who prescribed Burow's solution and 1 per cent boric acid ointment locally in addition to four x-ray treatments to the hands, one to the arms, and one to the eyelids. In spite of this therapy and the use of rubber gloves in her work, the rash continued to spread. A patch test was made with isopropyl alcohol and reported as negative.

This patient had a history of migraine of several years' duration, chronic nasal catarrh, and urticaria. None of these was aggravated during the period of her skin manifestations.

One per cent streptomycin patch test was positive in twenty-four hours with erythema 45 mm. in diameter, but there was no swelling or edema. Small vesicles could be seen dotting the entire patch area. Two weeks after the test was made the redness had faded somewhat but was still clearly demarcated. There was no increase in pruritus in the affected areas nor did any area break down after the patch was applied. On retest, using 0.1 per cent streptomycin, the patch test was negative. On this treatment the patient improved markedly and within two weeks was symptom free, although she continued to handle streptomycin syringes (using rubber gloves).

CASE 2.—A. S., a 35-year-old nurse, reported to the Allergy Clinic on Feb. 11, 1948, complaining of rash on the left hand and arm. She stated she had never had any rash previously. The present eruption began about Dec. 12, 1947, at the base of the second finger and from there spread to the interdigital spaces and other fingers of the left hand.

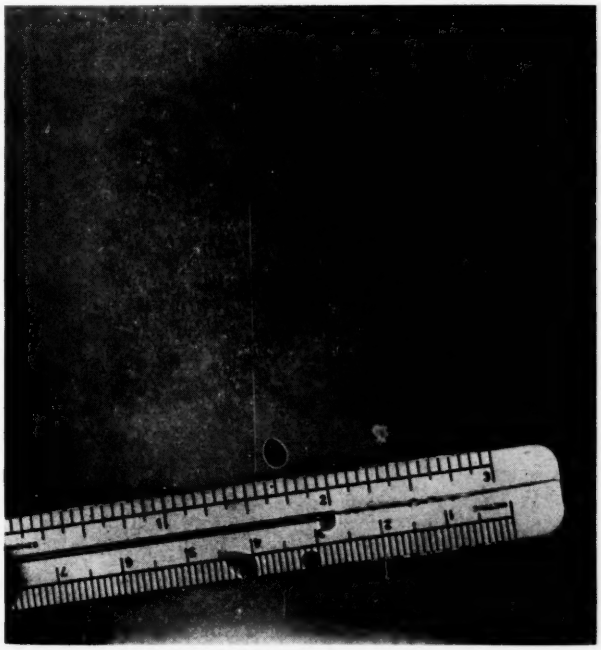

Fig. 1.—Case 2. Remaining erythema from patch test with 50 per cent streptomycin applied six weeks previously. Inside forearm.

At the onset there was also some rash on the eyelids and neck, but none on the face. The nurse had been in contact with streptomycin almost daily since June 22, 1947. She noted improvement on not handling streptomycin for four weeks during December, 1947, and January, 1948. She was also in contact with alcohol, face powder, rouge, lipstick, face cream, hand lotion, and shampoo. Her rash, at onset, was similar to that described in

Case 1. Patch testing at onset was done by her with a piece of rubber glove, glove powder, and with 50 per cent streptomycin. The tests with glove latex and powder were negative, but the large response to streptomycin still was present six weeks after it was applied[4] (Fig. 1).

The 50 per cent streptomycin was applied on a piece of gauze one-half inch square. This resulted in an erythema two inches square and caused an acute exacerbation of the lesions already present. Only the left eyelid was swollen and red previous to the test, but immediately following both eyes were so affected.

This patient had no allergic history with the exception of once having had hives. On treatment described for patient in Case 1 but omitting x-ray therapy she improved markedly and within one week was symptom-free although she continued to handle streptomycin syringes (using rubber gloves). Improvement also occurred when the patient remained away from work. One per cent streptomycin patch test was positive in twenty-four hours with erythema 20 mm. in diameter, slight edema, and multiple small vesicles. The redness faded within two weeks after the test was made but was still clearly demarcated as was the original test made with 50 per cent streptomycin. The affected areas had healed before the patch test was applied and no breakdown resulted from it although the former patch area became redder and itched for twenty-four hours. On retest, with 0.1 per cent streptomycin, the patch test was positive at twenty-four hours with itching and swelling, and erythema one inch in diameter. The erythema faded about one week after the patch was applied.

Case 3.—I. S., a 48-year-old female clinic attendant, reported to the Allergy Clinic on Feb. 12, 1948, complaining of swelling and edema of the eyes and a macular rash of the cheeks, arms, chest, and neck with pruritus. Since the onset about Dec. 1, 1947, she had

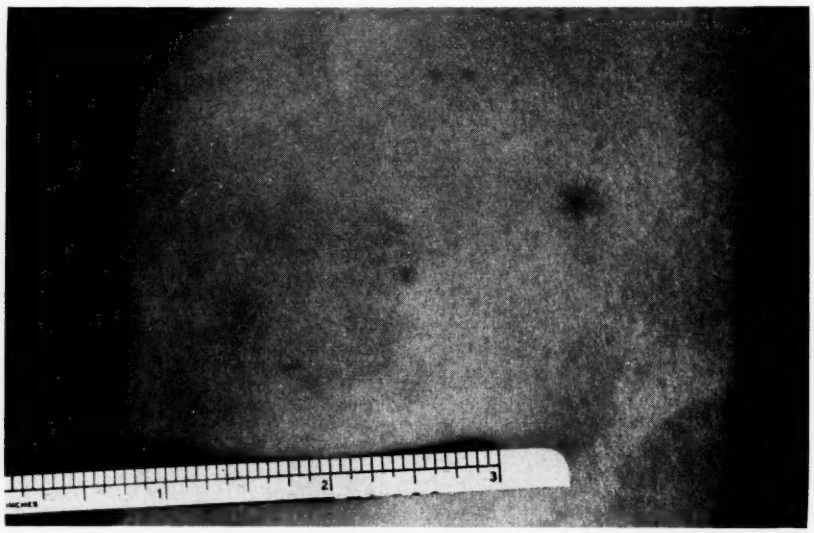

Fig. 2.—Case 3. Remaining erythema from patch test with 50 per cent streptomycin applied three weeks previously. Upper arm.

also noticed frontal headaches, pain in both eyes, and some difficulty in breathing through the nose. The symptoms did not all arise at the same time. Before external signs appeared the eyes began to itch. Shortly thereafter the rash appeared and about three weeks later swelling of the eyes was noted. She stated that she had never had a skin rash or swelling or itching of the eyes previously. She had handled streptomycin syringes almost daily since May, 1947. The rash at first appeared as small vesicles but when seen by me con-

sisted of red, dry, scaling macules. She uses no cosmetics of any kind and as far as she knows is not sensitive to any of the irritants with which she came in contact. She was treated by an outside physician with yellow oxide of mercury ointment to the eyes, Burow's solution to the rash, and Benadryl and ammonium chloride internally. The only improvement noted was a slight decrease in the swelling and itching about the eyes. Patch tests were made with glove powder and 50 per cent streptomycin. The former was negative and the latter resulted in a red, swollen, raised area one and one-half inches in diameter, containing many small vesicles which were still well demarcated after three weeks (Fig. 2). When first seen she had not worked for approximately a week and was slightly improved. She had a history of eczema and migraine which had occurred about twice a month for the last ten years. There was considerable improvement on treatment, and after a week, a patch test with 1 per cent streptomycin was applied. During the twenty-four hours the streptomycin was in contact, she noted sharp pains in that arm and soreness after about fifteen hours. The face became hot and swollen and the eyes swelled and itched even though the patient was on Pyribenzamine at the time. There was exacerbation and itching of the rash and deepening of the redness of the area of the 50 per cent streptomycin test. Because of the extensive reaction to 1 per cent streptomycin, no further patch tests were made on this patient. Both test areas were visible three weeks after the second patch was applied. Symptoms gradually diminished on treatment in spite of the patient's having returned to the same job.

Patch tests on eleven controls with various concentrations of streptomycin were all negative.

PROPHYLAXIS

The following procedure has been recommended for use by nurses and other personnel handling syringes which have contained streptomycin:

1. Rubber gloves are to be worn whenever syringes containing or having contained streptomycin are handled. Gloves so used are to be washed with tincture of green soap before being discarded and are not to be worn again before sterilization by boiling or autoclaving.

2. Streptomycin syringes after use are to be taken apart and placed in a receptacle with running warm water for fifteen minutes. The figure-of-eight tape placed with the syringe in the sterile packet is then to be used to keep the barrel and its respective plunger together.

3. All personnel who either administer streptomycin or handle the syringes which have contained it should be patch tested with 1 per cent streptomycin at least every two months.

If the preceding recommendations are followed, it is presumed that few cases of sensitization will occur.

TREATMENT

Although much has been written lately on the treatment of dermatitis venenata, the specific therapeutic measures to be followed in the disease due to streptomycin contact are quite definite. In the cases already described, several definite plans of treatment were prescribed by dermatologists and others. It was quite obvious that Thephorin, Benadryl,[4] x-ray treatments, Burow's solution, and calamine lotion were of little help. Results were obtained immediately and

cure in a short period of time, even while still exposed to streptomycin (though using rubber gloves), by the application of 2 per cent Pyribenzamine cream daily to the lesions themselves and administration of 50 mg. of Pyribenzamine internally four times a day. To further diminish irritation of the already irritated skin, Dermolate* was used in place of soap.

DISCUSSION

Originally the use of streptomycin in tuberculosis hospitals made this problem one which applied only to this type of institution. As further uses of the drug have developed and the supply has become more available, cases of streptomycin skin sensitivity in nurses and attendants will undoubtedly be reported from other hospitals. Needless to say, it is felt that if proper prophylaxis is carried out the suggestions given for treatment will be unnecessary. Apparently, even with almost daily handling of the drug, at least four months were necessary for sensitization in the cases reported. Properly spaced patch tests of exposed personnel should discover this sensitivity before actual lesions arise. The strength of solution used in the patch assumes importance because of the possibility of (1) systemic reaction or exacerbation of existing lesions, (2) false positives, and (3) the possibility of inducing sensitization by contact. Twenty-five and 50 per cent solutions are undoubtedly too strong, for positive reactions that occur when these strengths are used last for weeks. One-tenth per cent is too weak for it was found that even in a highly sensitive individual (Case 1), the result was negative. It would appear that the optimum strength of solution to be used in patch testing is 1 per cent. Of course, if systemic manifestations such as urticaria or angioneurotic edema are present, it would probably be wise to perform the first patch with 0.1 per cent streptomycin and if this is negative, to try 0.5 or 1 per cent. In the present series, the only time 10 per cent streptomycin patches were used was in testing individuals who were known never to have had contact with the drug in order to determine if this concentration had an irritant action on the skins of normal individuals.

Intradermal tests done with various strengths of streptomycin in our hands appeared to be unreliable in the more dilute solution and irritating in the more concentrated ones. It would also seem that the likelihood of inducing sensitivity or causing constitutional reactions was increased by such injection.

SUMMARY

1. Streptomycin sensitivity does exist and develops in certain individuals who have frequent contact with solutions of the drug. Apparently past or present allergy or penicillin sensitivity has no effect on the development of skin sensitivity to streptomycin.

2. The development of skin sensitivity to streptomycin is due to frequent contact over a period of several weeks. Just which individuals will be sensitized by this contact has not been determined.

3. A successful method of treatment and prophylaxis has been discussed.

*Manufactured by Rare Chemicals, Inc., Harrison, N. J.

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EFFECTS OF BENADRYL ON SYSTEMIC MANIFESTATIONS OF COLD HYPERSENSITIVITY*

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HYPERSENSITIVITY to cold or "cold allergy" was first reported in 1866 by Bourdon and numerous clinical reports have since been added. Horton and associates[3] have demonstrated that the symptoms are due to the release of histamine-like substances, as evidenced by such an action on the blood vessels, gastric secretion, and blood pressure. Wells and co-workers[8] have shown that histamine is antagonized by beta-dimethylamino-ethyl benzhydryl ether hydrochloride (Benadryl). Specific antagonistic action of Benadryl in subjects with cold hypersensitivity has also been noted.[8] A case of cold hypersensitivity is here reported. Observations are recorded on the vasomotor and gastric secretory changes prior and subsequent to the administration of Benadryl.

CASE REPORT

The patient, white, 25 years of age, had served as an Air Corps pilot for four years and was discharged on June 18, 1946. Just prior to separation, he first noticed that whenever he crossed his legs or bent his arms for short periods of time, he would experience numbness in the involved extremity.

A week prior to his admission to the hospital on July 3, 1946, while holding a piece of ice, he noted swelling, numbness, and tingling in the hands. This phenomenon was repeated when he held a glass of iced tea or a cold soda water bottle. When he ate ice cream or drank a cold beverage his lips tingled and swelled. A few minutes after entering an air-conditioned building his hands, feet, and legs felt as though "pins and needles were sticking in them." These symptoms disappeared upon emergence into warm air.

The patient had always been in unusually good health. There was a history of diphtheria at the age of seven years, measles at eight years, and a back injury in 1943. There were no complications. The patient had flown at high altitudes throughout his Army career and had never experienced any difficulties. He frequently went swimming and noted no unusual effects. A review of systems was essentially negative. He had never experienced hay fever, asthma, or other allergic manifestations. There was no familial history of hypersensitiveness or allergic phenomena.

Physical examination revealed a well-developed young man, not acutely ill. His temperature was 98.4° F.; pulse, 96; respiration, 15. Pupils were equal and reacted to light and accommodation. No changes were noted in the eye grounds. The mucous membranes of the nose and throat were slightly injected. The skin of the buttocks had acne and marked dermatographia was present. No wheezing or râles were heard in the chest. Blood pressure in the right arm was 120/78 mm. Hg; left arm, 112/74. Examination of the heart revealed no enlargement or murmurs. The liver and spleen were not paplable. The urogenital system showed no abnormalities and the skeletal system was grossly normal. Superficial abdominal, cremasteric, Achilles, patellar, triceps, and biceps reflexes were all present and equal. No pathologic reflexes were noted. There was no loss of vibratory sense and pain sensation was normal. No areas of hyperesthesia or hypesthesia were discovered. The peripheral vessels and pulsations were easily palpable and appeared uninvolved. Hyperabduction and hyperadduction of the arms were performed without discomfort.

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The blood showed a leucocyte count of 8,300 and the differential count consisted of fifty-five segmented polymorphonuclears, thirty-four lymphocytes, four monocytes, and seven eosinophiles. The hemoglobin was 17.5 Gm. and the sedimentation rate was 5 mm. corrected. The Kahn test was negative. The urinalysis was negative throughout. No cold agglutinins were demonstrated in the blood. X-ray of the chest revealed a normal cardiac silhouette and clear lung fields.

Special Tests.—Tests were performed by immersing the patient's hands and feet in water at 20° C. for five minutes. Room conditions were established so as to permit no cool or warm drafts on the subject. The blood pressure and pulse rate were recorded every fifteen minutes until a level base line was established. A stomach tube was introduced prior to the test and samples of gastric secretion were withdrawn at intervals. The amount of swelling was measured by immersing the right hand up to a definite line on the wrist into a container of water and recording the volume of water displaced. These tests were repeated several days later and at that time 400 mg. of Benadryl was administered during a period of twelve hours prior to the test; 150 mg. of this dose was given two hours before the test.

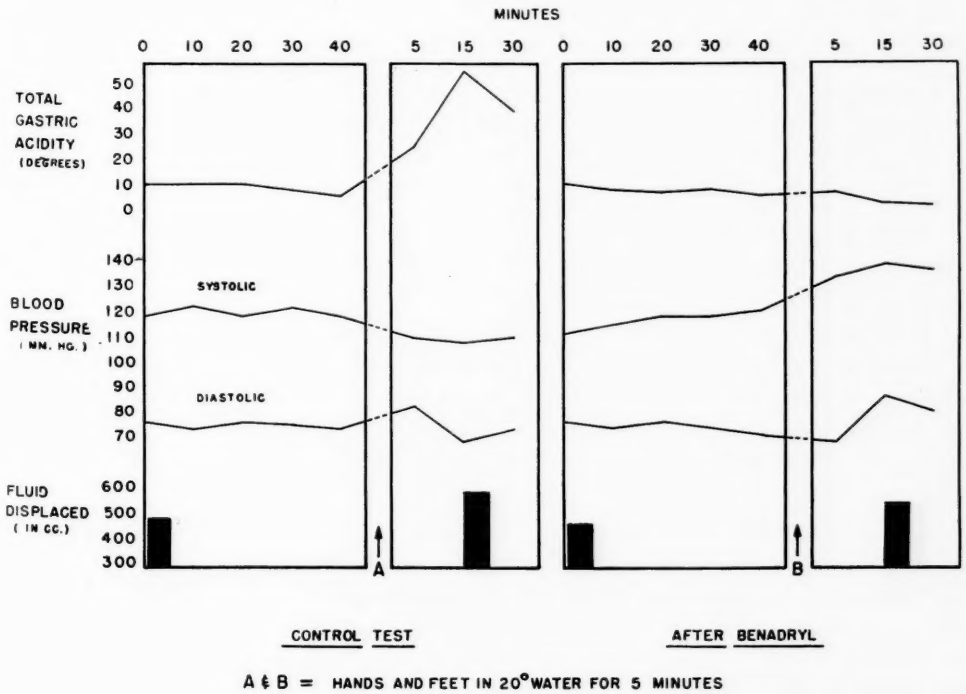
MINUTES
0 10 20 30 40 5 15 30 0 10 20 30 40 5 15 30
TOTAL GASTRIC ACIDITY (DEGREES)
50 40 30 20 10 0
BLOOD PRESSURE (MM. HG.)
140 130 120 110 100 90 80 70
SYSTOLIC
DIASTOLIC
FLUID DISPLACED (IN CC.)
600 500 400 300
A
B
CONTROL TEST
AFTER BENADRYL
A & B = HANDS AND FEET IN 20°WATER FOR 5 MINUTES

Fig. 1.—The effect of cold sensitivity on gastric acidity, blood pressure, and local tissue swelling before and after Benadryl.

When the patient's hand was plunged into the iced water, he promptly experienced marked tingling and numbness of the fingers. Redness and swelling of the hand were noted two minutes after withdrawing from the water. Nine hours later, the redness and edema disappeared but the patient continued to complain of stiffness in the joints. A few minutes after drinking cold water the patient complained of tingling of the lips. It was noted that they were swollen and reddened. Following the use of Benadryl, these subjective and objective symptoms were markedly lessened. The results are recorded in Fig. 1. It is noted that following the exposure to cold in the control period there resulted an increase of 100

c.c. in the volume of water displaced by the hand, a decline in systolic blood pressure of 10 mm. of Hg, a slight decrease in diastolic pressure, and a pronounced outpouring of acid gastric juice. After preparing the patient with Benadryl, the same cold stimulus resulted in an increase in water displaced of 67 c.c., a rise in systolic blood pressure of 20 mm. of Hg, a slight elevation of diastolic pressure, and no change in the secretion of acid in the gastric juice.

Treatment.—An effort was made to ameliorate the patient's symptoms by "desensitizing" him. He was placed on a regimen of two baths daily, the water covering everything but the head. The temperature of the bath water was 97° F. at first; this was gradually reduced over a four-week period until at the time of discharge he tolerated temperatures of 75°. In addition, 50 mg. of Benadryl and 5 mg. of benzedrine were given four times daily. The benzedrine was given to counteract the narcotic effect of Benadryl. Upon discharge the patient could eat ice cream and drink cold water with no ill effect. He was also able to withstand cold stimuli with minimal discomfort.

DISCUSSION

The mechanism of cold allergy has been explained in different ways. Horton and associates[3] are of the opinion that a chemical substance causing histamine-like reactions is produced in the skin upon exposure to cold; this, in turn, produces either local or general reactions. He felt that the symptoms could be reproduced by injection of histamine and stated: "Patients were unable to distinguish between systemic manifestations produced by immersion of the hand in cold water and those produced by subcutaneous injection of histamine."

Duke[1] believed that physical allergy due to cold or heat is a manifestation of a disorder in the heat regulating mechanism. He postulated that in some people this dysfunction is due to organic causes; in others, no pathologic process can be found except the history of a febrile disease preceding the onset of the hypersensitiveness.

Six cases of urticaria which were thought to be stimulated by emotion, by exercise, and by warming the body were described by Grant and co-workers.[2] This group believes that the urticaria resulted from the release of acetylcholine in the skin through the stimulation of cholinergic nerve fibers. The release of acetylcholine in turn leads to liberation of H-substances from skin cells.

Peters and his co-workers[6] observed a case of physical allergy to heat and exercise and noted the response to histamine and furfuryl trimethylammonium iodide (Furmethide), a substance which is chemically related to acetylcholine. They were able to prevent the urticaria produced by exercise and heat by injecting either atropine or epinephrine prior to testing. It was their opinion that both histamine and acetylcholine had to be present before the urticaria would appear since neither one alone would cause it in their patient. They concluded that while both histamine and acetylcholine were normally present in the cells, they were inhibited by enzymes. However, in hypersensitivity to physical agents these enzymes were reduced in amount.

The reversal of the histamine-like action of cold stimulation in my patient by an antihistamine agent is evidence in favor of the hypothesis that the chemical agent responsible for the symptoms is histamine.

Loew and associates[4] have shown that secretions from denervated gastric pouches in dogs following stimulation with histamine was reduced approximately 40 per cent by pretreatment with Benadryl. He concluded that it possesses a degree of specificity against histamine which approaches the specificity of atropine against acetylcholine. Thus, it can be expected that if cold stimuli release histamine-like substances which in turn cause the symptoms noted, good results may be anticipated with antihistamine agents. The effects of Benadryl on gastric secretion in my patient are similar to those observed by Loew in dogs.

Treatment of cold hypersensitivity has been directed in the main to "desensitization," using either histamine or exposure to progressively increasing doses of the offending physical agents.

Recently, antihistamine agents have been employed therapeutically. Torantil (histaminase) was used by Roth and co-workers[7] with beneficial effects in ten patients with hypersensitiveness to cold. Notier and Roth[5] employed Benadryl in a woman with cold allergy and noted temporary improvement.

SUMMARY

1. A case of cold hypersensitivity is reported in which the effects of cold on the blood pressure, gastric secretion, and local tissue were observed to be reversed after administration of an antihistamine agent (Benadryl).

2. The effects observed lend further support to the hypothesis that the release of histamine-like substances is responsible for the symptoms in this condition.

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AN EVALUATION OF CONJUNCTIVAL TESTING IN EXTRINSIC RESPIRATORY ALLERGY

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CONFIRMATORY skin tests are not obtained in about 10 per cent of patients with extrinsic allergic respiratory disease whose symptoms disappear completely under environmental control. This group of patients is made up of those with symptoms confined to one or more pollen seasons, those with winter symptoms only, and those with perennial symptoms with seasonal exacerbations.

Since positive eye tests, performed as described by Peshkin,[1] frequently confirmed pollen sensitivity, it seemed desirable to study the eye test reaction to house dust provided a nonirritating dry testing material could be prepared. After several preliminary attempts, the following procedure was found satisfactory for the preparation of dust powder.

Mattress, pillow, and funiture dust, obtained by means of a vacuum cleaner, was extracted and purified in accordance with the method of Efron and Boatner.[2] The material recovered after ammonium sulfate precipitation of the 25 to 75 per cent acetone precipitable fraction was dissolved in distilled water, dialyzed free of sulfate and vacuum dried from the frozen state. The recovered powder which was rapidly soluble in water and in tears was placed in the lower conjunctival sac of ten nonallergic persons and was found to be nonirritating. We next tested it in the conjunctival sacs of ten patients with immunologically specific house dust skin tests. In every instance the results of the eye test paralleled the skin tests. Since the substance induced positive eye tests in known house dust-sensitive people and negative eye tests in people not likely to be house dust sensitive, we felt that we could consider it specific and nonirritating.

Before extensive clinical studies of eye tests were begun, the following standards for interpretation were decided upon:

In a positive test, the eye must present a wedge-shaped area of injection and erythema extending from the conjunctival sac up to and involving the ciliary vessels. Associated with this, there must be some circumcorneal congestion. This is the minimal positive reaction and is marked 1+. The control test in the other eye, performed with an unreactive pollen, must be completely negative. Reactions were graded from 1+ to 4+, the latter reaction consisting of marked erythema, injection and edema of the sclera and conjunctiva.

In Table I are classified 490 patients with extrinsic allergic respiratory disease whose allergic disorders have been followed for many months. In 444 of these subjects, the skin tests confirmed the clinical impressions. In forty-six subjects, skin tests were not completely confirmatory.

The dust eye test confirmed the clinical course in thirty-seven of forty-six questionable cases occurring in a series of 490 patients with extrinsic allergic respiratory disease.

TABLE I. CLASSIFICATION OF PATIENTS WITH EXTRINSIC RESPIRATORY DISEASE

Skin tests confirm clinical impression		444
Skin tests not completely confirmatory of clinical impression		46
Some skin tests positive; Eye tests positive to other substances confirming clinical impression	14	
All skin tests negative; Eye tests positive to some substances confirming clinical impression	23	
Skin tests negative; Eye tests negative	9	
Total	46	

Table II shows the details of the study of the twenty-three patients with negative skin tests to pollens and dust in whom positive eye tests were obtained. The time of the year in which symptoms occurred, shown in the left side of the table, is correlated with the result of eye testing in each case. The dust eye test confirmed the clinical course in all but three subjects. In addition, the presence of pollen sensitivity was demonstrated in seventeen instances of ragweed and four of grass pollinosis.

TABLE II. NEGATIVE SKIN TESTS AND POSITIVE EYE TESTS IN SUBJECTS SUSPECTED SENSITIVE TO HOUSE DUST

	SYMPTOMS MANIFESTED IN				EYE TESTS IN RELATION TO SYMPTOMATIC MANIFESTATIONS			
						POLLENS		
PATIENT	SPRING	SUMMER	FALL	WINTER	DUST	SPRING	SUMMER	FALL
1			×	×	+	0	0	2+
2		×	×	×	2+	0	+	2+
3			×	×	+	0	0	+
4			×	×	+	0	0	+
5		×	×	×	+	0	+	4+
6		×	×	×	+	0	+	2+
7				×	0	0	0	2+
8	×		×	×	+	0	0	+
9			×	×	2+	0	0	+
10				×	2+	0	0	0
11				×	2+	0	0	0
12		×	×	×	2+	0	+	+
13			×	×	2+	0	0	2+
14			×	×	+	0	0	+
15			×	×	2+	0	0	2+
16				×	2+	0	0	0
17				×	2+	0	0	+
18			×	×	+	0	0	+
19			×	×	0	0	0	+
20			×	×	+			+
21	×		×	×	0			+
22			×		+			+
23					+			+

Table III is a detailed résumé of the fourteen patients with positive skin tests to some substances and positive eye tests to other substances to which the skin did not react. These latter allergens were shown to be significant from the clinical course of the disease. The time of the year in which symptoms occurred is shown on the left side of the table; the results of skin and eye testing on the right side. All fourteen patients gave a history either of fall exacerbation or were observed to have symptoms in the ragweed season. Ragweed sensitivity was confirmed only by eye test. In addition, two instances of tree and grass pollen sensitivity were confirmed.

TABLE III. PATIENTS EXHIBITING POSITIVE SKIN TESTS TO SOME SUBSTANCES AND POSITIVE EYE TESTS TO OTHER SUBSTANCES

PATIENT	SYMPTOMS MANIFESTED IN				TESTS IN RELATION TO SYMPTOMATIC MANIFESTATIONS						
					SKIN				EYE		
	WINTER	SPRING	SUMMER	FALL	DUST	SPRING	SUMMER	FALL	SPRING	SUMMER	FALL
1	×	×	×	×	+	0	+	0	+		4+
2	×			×	+	0	0	0			+
3	×			×	+	0	0	0			+
4	×			×	+	0	0	0	0	0	+
5	×			×	+	0	0	0	0	0	+
6	×				2+	0	0	0	0	0	2+
7	×				+	0	0	0	0	0	+
8	×		×	×	2+	0	0	0	0	+	+
9	×			×	+	0	0	0	0	0	+
10	×			×	+	0	0	0	0	0	+
11	×			×	+	0	0	0	0	0	+
12	×		×	×	+	0	4+	0	0		+
13	×			×	+	0	0	0	0	+	+
14	×		×	×	+	0	0	0	0	2+	2+

Patient 5 (Table III) is an interesting example of the usefullness of eye testing in confirming clinical impressions. This patient, a 26-year-old white woman, was first seen in November, 1945. At that time, she gave a history of winter asthma without any seasonal exacerbations. She was immunologically sensitive to house dust and was managed from that standpoint with excellent results until September, 1946. During the entire month of September she suffered from repeated paroxysms of asthma. Skin tests were repeatedly negative to ragweed extract in all strengths. After the ragweed season was over, we elicited a positive eye test to this substance. The patient was then treated with ragweed and house dust extract. During the ragweed season of 1947, she was entirely symptom-free.

Patient 12 (Table III) likewise is of interest from a different standpoint. This middle-aged man who had winter asthma with severe exacerbations in the grass season was treated for several years with house dust and grass pollen. He was discharged in September, 1945 and had no further manifestations until late August, 1947, when seizures of wheezing recurred. Skin tests at this time were strongly positive to house dust and grass pollen as they had been originally, but negative in all strengths to ragweed pollen. A ragweed eye test was, however, strongly positive. The new clinical sensitivity was confirmed by the latter test. The patient had returned for treatment because of the ragweed sensitivity and not because of the sensitivity indicated by skin tests.

SUMMARY

1. In forty-six of a series of 490 patients with extrinsic allergic respiratory disease, the clinical impression could not be confirmed by skin tests alone.

2. In thirty-two subjects skin tests were entirely negative.

3. In twenty-three of these, prepared dust and pollen powder were placed in their conjunctival sac, which tests confirmed the clinical impression completely and solved the problem of etiology.

4. Both skin and eye tests were negative in nine patients.

5. In fourteen subjects an additional sensitivity of clinical importance was demonstrated by opthalmic testing.

6. A dry material suitable for eye testing may be prepared by slight modification of the Efron-Boatner process.

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INTRANASAL APPLICATION OF BUFFERED TRIPELENNAMINE (PYRIBENZAMINE) SOLUTION IN ALLERGIC RHINITIS

A Preliminary Report*

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THE effects from the oral use of Tripelennamine† (Pyribenzamine) are now well known. Not known, however, are the effects from the direct intranasal use of this drug. We hereby report observations on eighty-one patients who were treated topically for allergic rhinitis with 0.5 per cent buffered solution of Pyribenzamine.

The effects of topical application of antihistaminic drugs to the skin have been reported by Friedlaender and Feinberg[1] who observed that such application inhibits whealing reactions to histamine more than when used orally. One of us (J. Z.)[2] has also observed that Benadryl, applied to the skin by iontophoresis, prevents whealing reactions to histamine applied subsequently. Friedlaender and Feinberg[1, p. 140] found a solution of 0.5 per cent Benadryl in physiologic saline too irritating when administered intranasally. One of us (J. B.) used a 2 per cent solution of Pyribenzamine for chronic perennial rhinitis with beneficial results. This strength, however, caused marked nasopharyngeal irritation which was eliminated when a 0.5 per cent strength was substituted. The administration of the weaker Pyribenzamine solution was then decided upon for our series. The purpose of this paper is to relate the effectiveness of this solution in the relief of nasal symptoms associated with allergic rhinitis.

METHODS

Selection of Patients.—This study was begun on July 24, 1947, just before the ragweed pollen season, and was continued through the ragweed season. The patients selected for the administration of this form of therapy were those who had symptoms in spite of treatment and those who had symptoms and had no previous treatment. They were both clinic and private patients. The age range was from 6 to 73 years. All had allergic rhinitis which we grouped for this study as follows: (1) Perennial allergic rhinitis; symptoms inadequately controlled by the usual allergic management, including orally administered antihistaminic drugs. (2) Ragweed pollen rhinitis; symptoms inadequately controlled with treatment including desensitization and orally administered antihistaminic drugs. (3) Ragweed pollen rhinitis; no previous therapy.

Dosage and Method of Administration.—A 0.5 per cent solution was used. A few patients had a momentary unpleasant sensation in the nose during the first few days but, in general, this dilution was well tolerated. However, three patients with chronic perennial rhinitis given a 2 per cent solution at the beginning of this study used this strength for five months with no apparent harmful

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†Supplied through the courtesy of Ciba Pharmaceutical Products, Inc., Summit, N. J.

effects. The dose recommended was 2 or 3 drops in each nostril every three or four hours, decreasing in frequency as improvement occurred. The usual allergic management was continued while the patient was under treatment, but oral antihistaminic drugs were omitted.

RESULTS

Evaluation of Results.—The evaluation of results was based on careful questioning of the patients during regular visits, usually at weekly intervals, before and after using the medication. Occasionally it was noted under direct observation that the use of the medication in the clinic or office resulted in relief of nasal obstruction within a few minutes. The results were considered excellent when symptoms responded promptly and quite completely to the medication; satisfactory when the symptoms were considered by the patient to be definitely improved, estimated at about 50 to 80 per cent relief; poor when the symptoms were only slightly or not at all improved.

TABLE I. SUMMARY OF CLINICAL RESULTS

TYPE OF ALLERGY TREATED	NUMBER OF PATIENTS	EXCELLENT RESULTS	SATISFACTORY RESULTS	POOR RESULTS
Perennial allergic rhinitis previously treated	16	8 (50%)	6 (37.5%)	2 (12.5%)
Ragweed pollen rhinitis previously treated	42	35 (78.6%)	6 (14.3%)	3 (7.1%)
Ragweed pollen rhinitis previously untreated	23	14 (61%)	9 (39%)	0

In patients with coexisting bronchial asthma, no appreciable change in the bronchial symptomatology was noted. In those with unilateral allergic polyposis, relief was obtained only on the side without the polyps. Upon using a vasoconstricting solution on the affected side prior to the Pyribenzamine solution, relief was obtained on that side also.

In most instances the nasal mucous membranes were examined before topical therapy was applied and again during treatment. In the majority of patients the mucous membranes appeared drier and the inferior turbinates took on a pinkish and more normal appearance with this therapy.

Untoward Effects From Pyribenzamine Solution.—Two patients were unable to tolerate this medication. In one the symptoms were aggravated; in the other it caused "pain in the head." In a few patients slight burning or stinging in the oropharynx and occasionally mild paroxysms of sneezing occurred when the Pyribenzamine solution was first introduced in the nose. However, these symptoms were transitory and were followed by clearing of the nose. In no patients were serious objective or subjective adverse effects noted. Drowsiness did not occur.

Duration of Relief.—The relief obtained from the use of this therapeutic agent seemed to last from one to twenty-four hours. The longer the medication was employed, the better the results and the less frequent was its use necessary. Thus the average patient who began by using it every four hours for about

five to seven days needed its help thereafter only once in twelve to twenty-four hours. After the prolonged use of this solution the clinical improvement sometimes lasted several weeks after treatment was stopped. Two patients who had excellent results for four weeks were then given only saline solution as a placebo and continued to feel well for three weeks. When symptoms returned, while using the saline solution, Pyribenzamine solution was substituted and again gave relief.

DISCUSSION

The two properties most likely responsible for the local activity of the Pyribenzamine solution are its antihistaminic and local anesthetic effects.[3] The direct application of the drug to the reacting hypersensitive nasal mucosa probably results in a greater local concentration of the drug where it is most needed than when the oral route is used and accounts for its more rapid effect.

A great advantage in the topical application of this drug is the lack of drowsiness so frequent with the oral use of antihistaminic agents. For example, one of our patients, a student who refused to take Pyribenzamine by mouth because it made him drowsy, obtained complete relief with the topical application of the solution. Similarly, a truck driver who obtained good results with oral Pyribenzamine wanted to stop the drug because it made his driving unsteady. He obtained excellent results with the topical application of the solution and had no side effects.

In some patients it was noted that Pyribenzamine instilled intranasally brought relief when vasoconstrictors gave insufficient or no relief. Thus, two children (aged 9 and 11 years) with allergic rhinitis, who developed severe headaches associated with almost complete bilateral nasal obstruction, unaffected by the ordinary vasoconstrictors, were relieved of their headaches within about an hour after the topical application of the Pyribenzamine solution.

Another advantage in the intranasal application of the solution is the absence of the rebound phenomenon, frequently observed with the usual vasoconstricting nose drops or nasal sprays.

In several instances this solution was tried in acute upper respiratory infections without apparent benefit.

SUMMARY

1. In eighty-one patients with allergic rhinitis treated with the topical application of buffered Pyribenzamine solution over 90 per cent obtained satisfactory or excellent results.

2. Early in the study a 2 per cent solution of Pyribenzamine was used but was soon replaced with a 0.5 per cent solution because the stronger solution caused local discomfort.

3. No serious local or general effects were noted.

4. Topical therapy was effective when antihistaminic drugs orally administered could not be tolerated because of drowsiness and other distressing symptoms.

5. The local effect is temporary and the usual allergic diagnostic and therapeutic measures are necessary.

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American Academy of Allergy

FIFTH ANNUAL MEETING OF THE ACADEMY
CHALFONTE-HADDON HALL, ATLANTIC CITY, N. J.
DEC. 6-9, 1948

SYNOPSES OF PAPERS

THERAPEUTIC BRONCHOSCOPY IN BRONCHIAL ASTHMA

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The records of 155 therapeutic bronchoscopies performed on 112 cases with bronchial asthma are reviewed. The results obtained and the technique appropriate for the asthmatic patient are presented. The following conclusions are reached:

1. The chief indication for bronchoscopic therapy in bronchial asthma is the state of emergency which arises from the patient's inability to expel the characteristic thick, tenacious mucus which might lead to asphyxiation. In a moribund asthmatic, therefore, bronchoscopy is as obligatory a procedure as the removal of a foreign body from a bronchus or a tracheotomy in a patient about to asphyxiate from diphtheria.

2. Other purposes of bronchoscopic treatment are the breaking up of a chronic state of asthma regardless of its severity, the relief of atelectasis, the dilatation of cicatricial bronchostricture and, occasionally, the relief of bronchiectasis resulting from asthma.

3. This treatment is not effective in asthma of short duration, in asthma associated with allergic shock, and in other instances where there is no clinical evidence of mucous plugs.

4. There are certain hazards in performing a bronchoscopy in allergic patients due to sensitivity to, and intolerance of, anesthetics and other medications administered before, during, and after bronchoscopy. This calls for certain precautions with respect to preoperative and operative drugs which are described.

5. If infection appears to be the dominant cause of the attacks, antibiotics may be introduced into the bronchi after the lavage. Measures to avoid reactions from sensitivity should be employed.

THE USE OF VASO-CONSTRICTORS AND VASO-DILATORS IN ASTHMA

ALAN G. CAZORT, LITTLE ROCK, ARK.

Routine blood pressure readings before and after giving epinephrine and aminophyllin have revealed a definite relationship between these readings and therapeutic response.

The physiology of the pulmonary circulation is discussed, and the importance of pulmonary congestion as a factor in dyspnea is emphasized. It is shown that epinephrine tends to increase pulmonary congestion while aminophyllin decreases it. These drugs do not act similarly in relieving asthma. It is further shown that peripheral blood pressure readings are an index of pressure in the pulmonary circuit, which itself has no appreciable vasomotor function.

A few illustrative case histories are cited.

It is concluded that the clinical indications for epinephrine and aminophyllin are clearly defined and readily determined.

AN APPROACH TO THE PROBLEM OF "EPINEPHRINE-FASTNESS"

FREDERICK F. YONKMAN, SUMMIT, N. J., AND MAURICE S. SEGAL, BOSTON, MASS.

The important problem of "epinephrine-fastness" can be attacked from a new angle as a result of the important work of Staub (Schweiz. med. Wchnschr. **76**: 818, 1946) and Farrerons-Co. (American College of Allergists Fourth Annual Session, March 12, 1948, to

be published) which demonstrates that intravenous epinephrine releases histamine or histamine-like substances, probably as a compensatory mechanism. This could in part explain the rapid return of elevated arterial tension to normal.

The release of histamine by epinephrine could also account partially for marked pulmonary edema observed in rabbits by Hallion and Nepper (J. de physiol. et de path. gén. **13**: 887, 1911).

If histamine or similar substances were released by epinephrine in the "epinephrine-refractory" asthmatic patient, successive injections of epinephrine could aggravate such a condition by additional release of histamine. Epinephrine's bronchodilating action might be defeated by the marked contractile action of histamine and likewise, epinephrine's vasoconstrictor and antiedemic actions could be negated by histamine's vasodilating and edemic propensities, both features resulting in aggravation of symptoms. If these conditions prevailed, antihistaminic therapy would seem to be indicated. Since such therapy has been found to be effective clinically in this situation, the conclusion would seem to be warranted that epinephrine-fastness represents histamine toxicity which predominates in the so-called target organ.

LOCAL NASAL THERAPY WITH PYRIBENZAMINE IN SEASONAL AND NONSEASONAL HAY FEVER

EMANUEL SCHWARTZ AND HARRY LEIBOWITZ, BROOKLYN, N. Y.

The paper presents the results of the clinical evaluation of the topical application of ½ per cent isotonic buffered solution of Pyribenzamine hydrochloride in seasonal and nonseasonal hay fever. In a group of ninety-five patients, complete or partial symptomatic relief occurred in forty-nine of fifty-nine, or 83 per cent of cases of seasonal hay fever, and in twenty-seven of thirty-six, or 75 per cent of cases of nonseasonal hay fever. No relief was obtained in nineteen cases, or 20 per cent of the patients in this group. None of the patients experienced any systemic toxic reactions. Thirty-eight, or 40 per cent of the group, complained of slight burning of the nose. Two patients were unable to tolerate the solution after the first application because of marked burning of the nose and throat.

In consideration of the results reported here, and in view of the absence of systemic toxic reactions, ½ per cent Pyribenzamine appears to be a valuable antihistaminic drug for topical nasal application in seasonal and nonseasonal hay fever.

IONTOPHORESIS OF PYRIBENZAMINE HYDROCHLORIDE IN NASAL ALLERGY

MERYL M. FENTON, DETROIT, MICH., AND ELSTON R. HUFFMAN, ELOISE, MICH.

The antihistamine drugs have been administered by various routes with great success in the control of allergic manifestations, in which release of histamine plays an important role. Iontophoresis of Pyribenzamine which results in migration of ions after application of a galvanic current has been used successfully for pruritic dermatoses.

This method was applied in the present study in patients with nasal allergy. Twenty patients with seasonal and perennial hay fever were treated by iontophoresis using a nasal pack saturated with PBZ in 2 per cent and 5 per cent solutions. Treatments averaged eight minutes to each side of the nose with the current varying from three to seven milliamperes in intensity. Half-wave galvanic current was used. Reactions consisted of slight temporary dizziness in 50 per cent, and drowsiness in 20 per cent of treatments given. Controls included distilled water or normal saline. Treatments were given twice a week.

Eight seasonal hay fever patients, who had not received previous specific therapy, were treated, with excellent results in six cases. Immediate improvement resulted in all instances, lasting eight hours in two and forty-eight hours in the others. Those with nasal obstruction were especially grateful for relief obtained. After two or three treatments these patients were then given specific therapy for the remainder of the season. Eleven cases with perennial hay fever were treated similarly for periods of four to eight weeks. Previous treatment had been of no value. Five cases or almost one-half of the total number received seventy-five per cent benefit. Two cases relapsed within two weeks, but the

remaining six still show improvement. One case of rhinitis medicamentosa due to Privine was kept comfortable when the nose drops were discontinued. One patient with rhinitis, nasal polyps, and bronchial asthma showed no improvement.

Iontophoresis offers a new approach for quick relief in seasonal hay fever, temporary relief in perennial hay fever, and the only relief for the patient who must discontinue nasal vasoconstrictors to which he has become sensitized.

NASAL USE OF ANTIBIOTICS IN RESPIRATORY ALLERGY

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Infections of the upper respiratory tract are clearly associated with respiratory allergy. Gram-positive organisms usually predominate. Penicillin and streptomycin have a marked capacity to induce sensitization. In a controlled study, tyrothricin 0.02 per cent did not lessen the incidence of allergic manifestations. Bacitracin 200 u m/1 was then studied and results were compared with control groups.

Cultures showed that in vitro the majority of organisms were sensitive to bacitracin. The sensitivity of the bacteria in the order of their occurrence was as follows: staphylococcus (variable sensitivity), diphtheroids (sensitive), Gaffky-Neisseria (sensitive). Other organisms, except gram-negative bacilli, were mainly sensitive. The large number of diphtheroids isolated was due probably to the richness of the media and the length of the incubation time.

In the clinical evaluation of results, hours of mild, moderate, and severe symptoms were recorded. The Negro bacitracin-treated group showed a definite trend to a lesser number of hours of symptoms per patient per week, whereas a smaller number of white patients did not show the same trend. It was, however, felt that the white patients were not well divided, and that they were possibly less cooperative.

In the small group studied there was no regular correlation between sensitivity of the organisms to bacitracin and the clinical response to therapy. However, the trend of clinical results is impressive enough to warrant further investigation. Clinical sensitization to the preparation was not observed.

FIXED ANAPHYLACTIC SENSITIZATION

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A paravascular injection of a 5 per cent Bromsulphalein solution caused a fixed-wheal type sensitivity to the compound over a circumscribed area of the right arm. Sensitization was discovered on intravenous reinjection of the dye one month later, which was accompanied by generalized shock symptoms.

Specificity of the sensitization was tested by the use of other halogen analogues of the compound; quantitative measurements of nonspecific reactivity were carried out. The significance of the findings for the mechanism of tissue-fixed reactions is discussed.

EXPERIMENTAL SENSITIZATION WITH FOOD DYES AND CROSS SENSITIZATION TO PARAPHENYLENEDIAMINE

R. L. MAYER, SUMMIT, N. J.

Baer, Leider, and Mayer have shown that many patients sensitive to paraphenylenediamine show cross sensitization to certain azo dyes commonly used as food colors, although these azo dyes, tested for sensitizing capacity on the skin of guinea pigs, were found to produce no sensitizations (Calvery). We have shown that, as in humans, certain of these dyes are capable of eliciting allergic reactions in guinea pigs sensitized to paraphenylenediamine, although they are not sensitizers or possess this activity in slight degree. One therefore must distinguish between substances which are able to sensitize and to elicit allergic reactions as well, and substances which are able only to elicit reactions in individuals previously sensitized with a related sensitizer. Certain of these "eliciters" may be as dangerous as the sensitizers themselves.

CLINICAL AND EXPERIMENTAL STUDIES WITH ORTHOXINE IN THE TREATMENT OF BRONCHIAL ASTHMA

JOHN J. CURRY, J. E. FUCHS, AND S. E. LEARD, BOSTON, MASS.

Orthoxine (orthomethoxy-beta phenyl-n propyl-methylamine hydrochloride) is a new sympathomimetic compound which, like ephedrine sulfate, is an active bronchodilator when given by mouth but in contrast has relatively little pressor or central nervous system stimulating effect. In view of the obvious advantages of such a compound in the treatment of bronchial asthma, the following studies were made. In fourteen asthmatic patients, asthmalike attacks with reduction in the vital capacity were induced by the parenteral injection of methacholine and histamine. Orthoxine in 200 mg. doses and ephedrine sulfate in 30 mg. doses were compared by their ability to protect against the reaction to histamine and methacholine according to techniques previously described. Another group of twenty-one patients with bronchial asthma were given orthoxine and ephedrine sulfate and the clinical response and side reactions were noted.

In both groups of patients the effects of orthoxine and ephedrine sulfate were comparable but undesirable side reactions were not experienced after orthoxine.

In twenty-four subjects given 100 to 200 mg. of orthoxine only two showed a slight elevation in the blood pressure and tachycardia was not observed in any case.

It appears that orthoxine has a definite place in the management of mild bronchial asthma since it is an effective bronchodilator, is active when given by mouth, and has relatively little pressor or central nervous stimulating effect.

HYPERSENSITIVITY TO MERCUHYDRIN WITH POSITIVE SKIN TEST

MAXWELL L. GELFAND, NEW YORK, N. Y.

A female rheumatic cardiac in congestive failure received 2 c.c. mercuhydrin intramuscularly for eleven consecutive days without any untoward reaction. About one hour after receiving the twelfth injection she complained of heaviness in the chest, palpitation, and chills. There was a rise in temperature to 103° F. with profuse sweating. The following morning the patient recovered completely and another dose was given with a similar response. Ten days later the injection was repeated and the patient again complained of chills, oppression of the chest, and fainting, and a rise in temperature to 102.8° F. appeared. Reducing the dose of the diuretic to 1 c.c. in no way altered the anaphylactic-like reaction. Salyrgan, another mercurial, was substituted and well tolerated. Intracutaneous tests with mercuhydrin and salyrgan were negative. Serum of another individual treated with mercuhydrin asymptomatically was injected into this sensitive person intradermally and a positive reaction was elicited. Normal human serum was used as a control.

The fact that the signs of fever, tachycardia, chills, and sweating occurred after multiple, well-tolerated injections, seems to favor an anaphylactic-like reaction in which the preliminary administration of the drug sensitized the patient. The sensitizing antigen was probably a mercuhydrin-plasma-protein combination formed during mercuhydrin therapy and existed in the blood, the mercuhydrin acting as a haptene.

EVALUATION OF ELECTROPHORETIC METHOD OF SKIN TESTING

ABRAHAM WALZER AND HARRY GOLAN, BROOKLN, N. Y.

A series of ragweed-sensitive hay fever cases were tested by electrophoresis with a mixture of giant and dwarf ragweed to determine the influence of various factors in the technique. The most effective procedure was one employing a special small type of electrode, a current density of 0.5 milliamperes, a time of three minutes, and an application of two drops of 0.1 mg. N extract to the positive pole.

With this technique, antigen applied at the positive pole produced positive reactions in 86 per cent of cases and 71 per cent of these also developed positive reactions simultaneously at the negative pole. With saline, no reactions were obtained.

When antigen was applied to the negative pole, 33 per cent of cases reacted at this site and none developed reactions simultaneously at the positive pole.

The authors have not found that skin testing by electrophoresis is more preferable as an experimental procedure than the intracutaneous technique.

AN EVALUATION OF THE EFFECT OF RADIATION ON THE NASOPHARYNX IN ASTHMATIC CHILDREN

VICTOR L. COHEN, BUFFALO, N. Y.

Fifteen asthmatic children were treated with radium applications to the nasopharynx according to the method of Crowe and Ward. Specific treatment was not started in new cases or discontinued previous to radiation in the old cases. Antiasthmatic drugs were prescribed as needed. At each visit subsequent to complete radium treatment (usually four) the patient was scored according to the amount of medication required, number of days in the hospital for asthma, number of days of asthma reported, and physical signs present in chest.

These scores were arranged according to the days subsequent to the completion of treatment and the averages were graphed. The trend of the graph indicates that the group improved for seventeen weeks, resumed the original status from the seventeenth to the twenty-first week, and became worse subsequent to the twenty-first week following the treatment. Observations were discontinued at thirty-four weeks after the radium treatment. The average length of observation covered a period of twenty-seven weeks.

EXPERIMENTALLY INDUCED SPECIES SPECIFIC INSULIN RESISTANCE IN THE RABBIT

WILLIAM FRANKLIN AND FRANCIS C. LOWELL, BOSTON, MASS.

Twenty-four rabbits were injected repeatedly over a period of months with large quantities of commercial (beef and pork) insulin incorporated in an emulsion of Falba and mineral oil containing acid-fast organisms (Freund's adjuvant). Several animals developed a high degree of resistance to insulin. Diabetes did not develop, and small doses of human insulin caused a pronounced fall in blood glucose after the development of resistance to commercial insulin. The resistance was, therefore, species specific and apparently due to the development of antibody directed against beef and pork insulin.

Beef insulin treated chemically so as to destroy its hypoglycemic activity still retained its ability to combine with antibody, indicating that the resistance was not due to a true antihormone. Insulin-neutralizing antibody has been detected in the serum of at least one of the resistant animals. No allergic manifestations followed intravenous or endermal injections of insulin. Tuberculin type hypersensitivity has developed in some of the injected animals to both tuberculin and an extract of *M. butyricum*, the organism used in the adjuvant mixture.

A STATISTICAL STUDY OF THE EMOTIONAL COMPONENT IN ALLERGY; ITS RELATIONSHIP TO THE AGE AND SEX DISTRIBUTION OF 6000 CASES

MILTON B. COHEN AND LEWIS E. ABRAM, CLEVELAND, OHIO

We have studied the age-sex distribution in more than 6,000 allergy patients followed in private practice for an average period of eighteen months.

The study demonstrates that boys are affected twice as frequently as girls in the first decade. In the teens, the distribution is about equal, but between 20 and 35 years of age, there are twice as many females as males. This reversal makes up for the initial preponderance of males so that there is no significant distribution difference between the sexes in the entire series.

During the last three years, we have divided our patients into the following four groups.

GROUP I. Those with uncomplicated extrinsic allergy.

GROUP II. Those with extrinsic allergy in whom satisfactory results were not obtained until various emotional problems were solved.

GROUP III. Those with typical intrinsic allergy or with organic complications of extrinsic allergy.

GROUP IV. Those with symptoms like allergy, without the characteristic findings of allergy, in whom the symptoms represented organ neuroses.

When we studied the group, age, and sex distribution of 653 patients followed during 1947, it was found that the females in Groups II and IV were responsible for the increase in incidence for females between the ages of 20 and 35. Charts and curves of these distributions will be shown. They give statistical evidence of the importance of the emotional component in allergy.

GROUP PSYCHOTHERAPY AS AN AID IN THE TREATMENT OF ALLERGIC SYMPTOMS

HYMAN MILLER AND DOROTHY W. BARUCH, PH.D., BEVERLY HILLS, CALIF.

For the past three years the authors have conducted a group in psychotherapy. The varying membership of the group has averaged about twenty individuals quite evenly divided between men and women and allergics and nonallergics.

The procedures of group psychotherapy have been described and the outcomes with allergic patients have been pointed out in previous publications. As in individual psychotherapy, association, projection, release, and increased insight play important roles. The present paper deals with the use of graphic material, patients' drawings, and paintings to illustrate the typical functioning of the group.

When members of the group spontaneously brought in drawings and paintings depicting their dreams, phantasies, or other unconscious materials to the meetings of the group, various members associated to them projected their feelings into them, evidenced release, and often showed increased insight into the meaning of their allergic symptoms.

Lantern slides of several pictures by allergic members of the group are here presented. How the pictures were used both by the originators and by other allergic members is discussed. The value of group therapy as exemplified by this technique is pointed out by a brief discussion of the psychodynamics involved.

OBSERVATIONS ON THE EFFECT OF ADRENOCORTICOTROPHIC HORMONE ON A CASE OF LOEFFLER'S SYNDROME, AND A CASE OF TROPICAL EOSINOPHILIA

P. HERBERT AND B. ROSE, MONTREAL, CANADA

In early observations on the effect of the administration of adrenocorticotrophic hormone to man, Thorme et al. noted that among the changes produced, a decrease in the percentage of eosinophiles occurred. For this reason it was thought to be of interest to observe the effect of A.C.T.H. (Adrenocorticotrophic hormone) administered to cases in whom a high eosinophile count is found. Two such cases were available for study. The first was a case of Loeffler's syndrome, and the second one of tropical eosinophilia. In both of these a high persistent eosinophile count was encountered.

The administration of A.C.T.H. to the case of Loeffler's syndrome resulted in a rapid virtual disappearance of the eosinophiles within six to eight hours. In addition, the transitory infiltrations in the chest as shown by x-ray were cleared up within twenty-four to forty-eight hours, and a complete remission of symptoms occurred. By contrast, the administration of A.C.T.H. in the same dosage of the case of tropical eosinophilia resulted in little change in the blood picture. There was no alteration of clinical signs or symptoms. This case, however, was subsequently treated with arsenicals and resulted in complete remission.

FACTORS INFLUENCING REAGIN FORMATION IN EXPERIMENTAL HUMAN SENSITIZATION TO ASCARIS LUMBRICOIDES ANTIGEN. IV. THE INFLUENCE OF A PREVIOUS SENSITIZATION ON RATE OF REAGIN FORMATION

ELOISE W. KAILIN, ELINOR A. ROSSBACH, WASHINGTON, D. C. AND MATTHEW WALZER, BROOKLYN, N. Y.

Patients at a psychiatric hospital who had previously been artificially sensitized by injections *Ascaris lumbricoides* antigen and who had lost their cutaneous sensitivity were resensitized to this antigen approximately two years after the first procedure The reagin-antibody

response in these subjects was more rapid on resensitization than on the original stimulation and was also more rapid than the response of control subjects being sensitized for the first time.

The duration of artificially induced sensitivity in the first series was found to be less than two years in over 80 per cent of thirty-two persons under observation. The average time for the appearance of cutaneous sensitivity on resensitization was approximately one-third as long as original sensitization of the same subjects. Previously observed differences in the rate of sensitization, attributed to either racial or sex factors, were not observed in this study. The possible causes for the discrepancies in the results of the two series are discussed.

THE EFFECT OF PYRIBENZAMINE AND/OR RUTIN ON INVERSE ANAPHYLAXIS, FORSSMAN ANAPHYLAXIS, AND ACTIVE ANAPHYLAXIS

CARL E. ARBESMAN AND ERWIN NETER, BUFFALO, N. Y.

I. Antiguinea pig-rabbit serum was prepared by injecting rabbits three times weekly with guinea pig serum. The rabbits were bled at the end of four weeks. High precipitin titer was obtained. This antiguinea pig-rabbit serum was then injected intracardiacally into guinea pigs. The least amount of serum which would produce death in the guinea pigs was determined.

Guinea pigs were given varying amounts of rutin and/or Pyribenzamine fifteen to sixty minutes prior to the injections of antiguinea pig-rabbit serum intracardiacally. No protection against inverse anaphylaxis was obtained from either rutin, Pyribenzamine, or a combination of both.

II. Rabbits were injected with suspension of sheep's cells producing antisheep's cells rabbit serum (Forssman's antibody-amboceptor). The same experiment as above using this antibody intracardiacally in the guinea pig was performed.

III. Guinea pigs were sensitized with normal rabbit serum. Three weeks later these were shocked with intracardiac injections of normal rabbit serum. The effect of rutin and/or Pyribenzamine on the prevention of acute anaphylactic shock was determined. Rutin alone did not prevent against anaphylaxis, even in massive doses.

IV. Pathologic studies of guinea pigs that died in acute anaphylaxis, inverse anaphylaxis, passive anaphylaxis, and histamine shock will be presented and compared.

AIR-BORNE ALLERGENS IN THE NATIONAL PARKS

OREN C. DURHAM, PH.D., CHICAGO, ILL.

Acadia: The trees, grasses, and ragweeds of the New England area. More ragweed pollen than in upper New England.

Bryce Canyon: Wind-pollinated plants are rare, ragweeds absent.

Crater Lake: Total count of all pollens during summer and fall (except pine) was 87, 9 ragweed.

Everglades: The ragweed incidence is probably less than at Miami. Grasses are likely the most important factor.

Glacier: Ragweed, sagebrush, and Russian thistle pollens are practically absent during the tourist season.

Grand Canyon: Chief hazard is from sagebrush. Extremely little ragweed.

Grand Teton: Sagebrush is abundant. No ragweeds.

Great Smoky: A large variety of trees, the common grasses, and a fair amount of ragweed.

Hot Springs: Ragweeds, trees, and grasses are abundant.

Isle Royale: Isle Royale offers the best ragweed refuge of the north central states.

King's Canyon: No important hay fever weeds and no appreciable acreage of grasses.

Lassen Volcanic: During July, August, and September the total count of all pollens 47, 3 ragweeds.

Mammoth Cave: Well within the ragweed area. Heavy ragweed pollen incidence, also much tree and grass pollen.

Mt. McKinley: All Alaskan studies suggest total absence of ragweeds.

Mt. Ranier: Active allergenic pollens almost wholly absent except for grass at Longmire Station.

Olympic: Plaintain is chief offender. Total seasonal ragweed count, 4.

Platt: Visitors encounter large amounts of all central western hay fever pollens.

Rocky Mountain: Sagebrush is the chief offender. Much less at Grand Lake than at Estes Park.

Sequoia: 3 ragweed pollen grains in three months, 7 sagebrush.

Yellowstone: Grass and sagebrush fairly abundant. Practically no ragweed.

Yosemite: Incense cedar in winter, oak in spring, *very* small amount ragweed and sage in late summer and fall.

Zion: Very small amount of ragweed during spring, trees in fall. Shadscale and oak heaviest producers.

SPECTRAL STUDIES OF ANTIHISTAMINIC DRUGS

ALEX S. FRIEDLAENDER, SIDNEY FRIEDLAENDER, AND J. M. VANDENBELT, DETROIT, MICH.

This study concerns itself with the effect of antihistaminic drugs on the erythema normally produced by certain wave lengths of the spectrum. Spectral absorption studies were carried out with thirteen compounds currently available, using the Beckman Quartz Spectrophotometer. These data indicate that Pyribenzamine, Thenylene, Neo-Antergan, Pyrollazote, Tagathen, Neohetramine, and Antistine possess absorption bands in the region of 2950 to 3150Å, and should be effective as screening agents in preventing the erythema of sunburn. Clinical studies show that erythema from ultraviolet light is prevented when the aforementioned drugs are applied either to the skin or to appropriate filters interposed between the light source and the skin. Benadryl, Trimeton, Decapryn, Thephorin, C5581H (Bristol), and AH 853 (Parke-Davis) show no absorption bands in the region of the sunburn spectrum, and are found on clinical study to exert little effect in preventing the development of erythema from exposure to such wave lengths. This would indicate that the sunburn-protecting effect of the first group of drugs is largely related to their screening properties. Experiments were also carried out to determine the extent of any antihistaminic action involved. Comparisons were made with other agents known to possess sun-screening properties.

ASTHMA CAUSED BY HALOGENS

ROBERT G. LOVELL AND JOHN M. SHELDON, ANN ARBOR, MICH.

Bronchial asthma caused by one or more of the halogens is a rare condition, although other allergic manifestations due to this group are not uncommon. A case is presented in which bronchial asthma followed the application of iodine, topically and in connection with cholecystography. Asthma also occurred when the patient encountered chlorine in a laundry compound and in the municipal drinking supply. The methods of diagnosis and the clinical significance of this type of halogen sensitivity in regard to dietary manipulation and other diagnostic procedures are discussed.

FURTHER OBSERVATIONS ON PREVENTION AND TREATMENT OF RESPIRATORY INFECTIONS IN ALLERGIC PATIENTS

SAMUEL J. PRIGAL, NEW YORK, N. Y.

Approximately 400 patients have been treated with a variety of aerosols, more than half of whom were definitely allergic individuals. These have been treated with two varieties of aerosols; antispasmodics, such as aminophyllin, epinephrine, and Isuprel, singly and in combination, and antibacterial agents such as sulfonamides (sulfadiazine, sulfacetimide), penicillin, streptomycin, and bacitracin.

Evaluation will be made of the dosage and number of treatments necessary to obtain satisfactory results in the treatment of infective asthma, sinusitis complicating allergic rhinitis, and other related diseases. A review will also be made of adverse reactions in particular to penicillin and streptomycin. A special review of children treated with aerosols will be emphasized, in which the author uses the so-called "bathroom treatment." The prophylactic aspect of aerosol therapy will also be evaluated.

CAPILLARY PERMEABILITY STUDIES WITH THE DERMOFLUOROMETER

LEO H. CRIEP AND MACY I. LEVINE, PITTSBURGH, PA.

Fluorescence in the skin can be determined by means of an instrument called the dermofluorometer. Using this instrument, we determined the natural skin fluorescence in normal and allergic patients. The fluorescence was then determined during a one-hour period following injections of fluorescein and curves were drawn for each patient. We then injected histamine, ragweed extract, and buffered saline and compared the fluorescence over the resultant wheals, following injection of fluorescein with the fluorescence over control sites. All patients had marked hyperfluorescence over the wheal sites. The effect of Pyribenzamine on the fluorescence following injection of fluorescein was then noted. Small doses of Pyribenzamine did not appear to have any effect. However, massive doses of Pyribenzamine appeared to reduce the hyperfluorescence over histamine wheal sites. Erythema was produced by means of hot or cold applications. Histamine wheals were produced in the erythematous site and fluorescence curves were made for the erythematous area, for the site of the wheal which was in the erythematous area and a control site and wheal in normal skin. Results consistently showed that the fluorescence was most marked over the site of the histamine. Wheal in normal skin and the fluorescence were least marked over the erythematous area. Fluorescence was approximately the same over the wheal which was in the erythematous area and over the normal skin itself.

BACTERIAL ALLERGY: A CRITICAL REVIEW. REPORTS OF EXPERIMENTS

OSCAR SWINEFORD, CHARLOTTESVILLE, VA., AND JAMES HOLMAN, DALLAS, TEXAS

Discussion of problems of antigens, antibodies, tissue responses, desensitization, diagnosis, etc.

Method of preparation of soluble antigenic fractions of bacteria.

Anaphylaxis and Schultz-Dale reactions with bacterial fractions.

Results of 3,860 tests with fractions of fourteen kinds of bacteria.

A STUDY OF SOLUBLE SPECIFIC ANTIBODY COMPLEXES

HERMAN N. EISEN AND FRED KARUSH, PH.D., NEW YORK, N. Y.

Within the past decade it has become apparent that the so-called "Unitarian" hypothesis regarding the relation between antigen and antibody requires modification, since, for a single antigenic determinant, it can be demonstrated that the specific antibody is a mixture of molecules of different reactivities. For example, some antibody molecules form precipitates with antigen, whereas others combine with the antigen to form soluble complexes. The distinction between precipitating and nonprecipitating antibodies is significant in human hypersensitivity since, in this state, the antigen-antibody complexes almost always are of the latter type, whereas in most immune states the complexes form precipitates. On the basis of the "lattice" theory of antigen-antibody precipitation it would be anticipated that the properties of antibody that are of critical importance in determining precipitability would be: (1) "valence," i.e., the maximum number of combining sites, and (2) free energy of binding of antigen to antibody. Experimental approaches to the clarification of these antibody properties have been indirect and subject to controversial interpretation. We have undertaken a direct approach to the study of this question, using purified antibodies and dialyzable homologous haptenic molecules. By equilibrium dialysis it is possible to investigate the binding of hapten by antibody. The results of such studies permit direct estimation of the antibody's maximum number of combining sites, the average free energy of binding at these sites, and the corresponding range in binding energies. Further applications of this approach may yield insight into the differences between immune and sensitizing antibodies.

THE CHEMICAL ISOLATION AND BIOLOGICAL ASSAY OF EXTRACELLULAR ANTIGENIC FRACTIONS FROM PATHOGENIC FUNGI

EDMUND L. KEENEY AND NILS ERIKSEN, PH.D., SAN DIEGO, CALIF.

Dry, crude protein and carbohydrate preparations have been obtained from two strains of *Trichophyton mentagrophytes* (a cause of dermatomycosis) and one strain each of *Trichophyton rubrum* (a cause of dermatomycosis), *Histoplasma capsulatum* (the cause of

histoplasmosis or Darling's disease), *Blastomyces dermatitidis* (the cause of blastomycosis), and *Candida (Monilia) albicans* (the cause of candidiasis or moniliasis). The fungi were grown on synthetic liquid media and in the cold. Proteins were removed either by precipitating with alcohol in the cold or with ammonium sulfate at room temperature. After the proteins had been removed, carbohydrate material was recovered by treating the filtrate with alcohol. A partial chemical characterization of these fractions will be presented.

Guinea pigs and rabbits have been infected intradermally with *Trichophyton mentagrophytes* and *Candida albicans* and the development of the anaphylactic and tuberculin types of hypersensitivity during the first infection and reinfection have been followed by skin testing and by the determination of precipitating antibodies. The development of the tuberculin type of hypersensitivity paralleled closely the course of the infection. The anaphylactic type of hypersensitivity appeared after the infection had reached its peak.

A fraction obtained by precipitating *Candida albicans* broth concentrate with 75 per cent alcohol proved to be a powerful sensitizer. This fraction was superior in sensitizing ability to a 33 per cent alcohol fraction and to the commercial preparation, Oidiomycin.

THE USE OF RAGWEED OINTMENT IN DETERMINING SEASONAL VARIATION OF OPHTHALMIC SENSITIVITY

MAURICE H. SHULMAN, BOSTON, MASS.

This investigation was initiated to determine the variation of ophthalmic sensitivity to ragweed pollen before, during, and after treatment with ragweed extracts. The disadvantages of using pollen extract for ophthalmic testing became evident. Simulating Nature, a preparation of whole ragweed pollen in a nonirritating ophthalmic ointment was prepared. One hundred patients were tested 591 times over a two-year period. Treated as well as untreated cases were included.

A large percentage of the cases tested showed a rapid diminution of eye sensitivity concomitant with a rising tolerance of pollen dosage.

Another group of patients showed an initial diminution of eye sensitivity followed by a fixation of eye sensitivity at a moderately high level. In these cases, pollen dosage was not tolerated above the level of fixation of the ophthalmic sensitivity.

A third group of patients showed positive ophthalmic reactions with high dilutions of pollen. Prolonged treatment caused no diminution of ophthalmic sensitivity. In these patients pollen dosage was poorly tolerated.

VITAL CAPACITY STUDIES TO DIFFERENTIATE TYPES OF BRONCHIAL ASTHMA

SIDNEY W. JENNES, WATERBURY, CONN.

The author introduces the subject with a short discussion of the history and technique of performing vital capacity studies. A series of 130 patients with bronchial asthma is divided into two groups: (a) extrinsic or nonbacterial, (b) intrinsic or bacterial. A study of the vital capacities of these patients reveals a marked difference in respiratory reserve between the two groups.

The effects of age, duration of the disease, sex, and other factors on the vital capacities are discussed. An interesting finding regarding age of onset in the intrinsic or bacterial group is noted. Charts are presented to illustrate the studies.

VEGETABLE GUMS BY INGESTION IN THE ETIOLOGY OF ALLERGIC DISORDERS

H. HAROLD GELFAND, NEW YORK, N. Y.

The purpose of this paper on the vegetable gums is to report the incidence of these allergens as sensitizing agents by means of ingestion. The vegetable gums as causative factors in allergic disturbances, by means of inhalation, surface contact, and injection, have been reported in a previous publication.

The various brands of food in which these gums are included (for the purpose of adding bulk, thickness, and binding qualities) are listed in the present paper, and allergic disorders in ten subjects resulting from the ingestion of these foods are reported in detail.

The series consists of seven males and three females, ranging from 11 to 55 years of age. Twenty-one types and brands of food, containing the vegetable gums karaya, tragacanth, and arabic, were ingested by these subjects, and resulted in the following allergic manifestations: bronchial asthma, allergic cough, itchy gums, gastrointestinal allergy, angioedema, urticaria, and vasomotor rhinitis. Of these ten subjects, seven reacted by positive skin tests to karaya, nine to arabic, and eight to tragacanth.

Results of clinical trial and ingestion experiments with the powdered gums taken in water, in addition to routine skin tests and passive transfer studies, are presented as evidence of the role of these gums by ingestion in the causation of the allergic disorders listed.

SUMMER ASTHMA MYSTERIES

HOWARD C. LEOPOLD, PHILADELPHIA, PA.

The paper presents a discussion of several of the unsolved problems of summer asthma with case examples. One problem discussed is that of seasonal, summer, nocturnal asthma, without hay fever, and without any evidence of sensitivity to air-borne pollens or molds. A possible primary cause of recurrent summer asthma is physical allergy to heat or the effect of climatic factors.

The second problem is that of cases of summer hay fever and asthma which clinically are apparently due to pollen sensitivity, primarily, and other allergens as synergists, but in which cases the pollen or mold sensitivity cannot be demonstrated by the available testing techniques, and the results of treatment are unsatisfactory.

EXPERIMENTAL COLLAGEN DISEASE FROM HYPERSENSITIZATION TO STREPTOCOCCUS TOXIN

LEO H. CRIEP AND LLOYD D. MAYER, PITTSBURGH, PA.

This paper deals with a series of experiments on rabbits in which the work of Rich and Gregory has been repeated and confirmed. Rabbits were also injected with streptococcus toxin intravenously with the resulting production of evidence of collagen disease. These lesions are found in the kidney, lungs, liver, and heart. These experiments were done because of the frequent association of collagen disease and evidence of hypersensitization in certain clinical conditions such as rheumatic fever and periarteritis nodosa.

ALLERGIC MANIFESTATIONS OF THE EYE AND SURROUNDING STRUCTURES

SAMUEL J. TAUB, MARY JANE FOWLER, BENJAMIN R. GUTOW, ROBERT E. MILLER, AND ROBERT G. TAUB, CHICAGO, ILL.

It is apparent that the role of allergy in diseases of the eye is assuming increasing importance in the practice of the general practitioner, ophthalmologist, allergist, and dermatologist. This is attested to by the voluminous additions to medical literature. Any tissue of the eyeball and adjacent structures may be involved in the allergic reaction. We shall discuss some patients who have come under our observation with various ophthalmological complaints including interstitial punctate keratitis, central chorioretinitic atrophy and other conditions of the eye in which an allergic etiology was manifest, and who responded, completely or partially, to allergic management.

The varied number of excitants and their mode of transmission to the eye and surrounding structures is discussed.

HYPOSENSITIZATION THERAPY IN PATIENTS WITH LOCAL AND CONSTITUTIONAL REACTIONS TO INSULIN

MERLE M. MILLER AND JOSEPH T. BEARDWOOD, JR., PHILADELPHIA, PA.

Over 200 cases of insulin sensitivity have been published, but a great many cases of allergic reaction to insulin are never recorded. In a series af fifty-three cases of insulin sensitivity we have had the opportunity of observing, thirty-nine had local reactions and fourteen had systemic reactions of varying degrees. Reactions to insulin vary in intensity from mild local sensitivity to anaphylactoid shock. Hypersensitivity to insulin can be induced as easily in nonatopic as in atopic individuals. Insulin may be a species specific

antigen or it may be a distinct specific antigen. Antibodies developed may sensitize or immunize the patient. There may be an immunologic similarity between insulin sensitivity and insulin resistance. Hyposensitization can be accomplished in a certain percentage of cases. Antihistamine drugs may be a valuable adjunct in therapy.

A schedule for hyposensitization to insulin is given, and slides of the test charts on these patients will be shown both by direct and passive transfer methods.

ALLERGIC TOXAEMIA AND FATIGUE DUE TO FOOD ALLERGY

ALBERT H. ROWE, OAKLAND, CALIF.

Since this syndrome was first described by me in 1930 our experience has amply corroborated its frequent occurrence. It also has been confirmed by Moreno in Buenos Aires and especially by Randolph in Chicago.

The analysis of seventy patients suffering with this syndrome due to food allergy will be presented. The relative frequency of fatigue, lack of energy, aching in the joints, muscles, and other parts of the body, restlessness and lethargy, inability to concentrate, irritability, confusion, depression, emotional instability, nervous tension, and insomnia will be charted. Other associated manifestations of allergy and their frequency will be shown. The information obtained from a carefully recorded dietary history will be analyzed. The occurrence of positive skin reactions to pollens, other inhalants, and foods will be presented. Other laboratory findings will be discussed. The writer's method of study of food allergy in these cases will be briefly reviewed.

MOLD COMMITTEE OF COUNCIL ON AEROALLERGENS

SPECIAL REPORT

By a special arrangement supported by a grant from the American Academy of Allergy, Dr. E. C. Stakman, Chief of the Division of Plant Pathology and Botany of the Department of Agriculture, University of Minnesota, University Farms, St. Paul, Minnesota, has agreed to give the following much needed help to members of the Academy:

1. Maintenance of a type culture collection of fungi which may be causes of allergic manifestations
2. Furnishing of cultures of any of these fungi to individuals on request
3. Aid in identification of fungi submitted by those who make surveys or those who have special need for such identification
4. Investigation in improvements in methods and special media for culturing special fungi.

It is urged that those who need help along the above lines make use of this service by contacting Dr. Stakman's laboratory. Full utilization of these aids can help materially in amplifying and improving the mold surveys of various localities and can be of material assistance in providing reliable sources of cultures for antigen production.

SAMUEL M. FEINBERG, CHAIRMAN.

ANNOUNCEMENTS

A course on Allergy and Immunology, sponsored by the American College of Physicians and approved by the Committee on Education of this Academy, will be held at Atlantic City, N. J., April 28 to May 1, 1949. Detailed plans of the course will appear in the March issue of the JOURNAL and may be had from E. R. Loveland, secretary of the American College of Physicians, 1700 Pine Street, Philadelphia, Pa.

The Committee on House Dust of the Section on Miscellaneous Inhalants of the Research Council of the Academy has plans for the chemical fractionation of house dust. These plans call for a large quantity of house dust—perhaps a ton.

We are requesting the Academy to send in house dust, as much as possible, ten to twenty pounds or more, if possible. This may be obtained with a vacuum cleaner from old mattresses, stuffed furniture, or pillows.

Please do not include ordinary dirt from floors or carpets, since this is too low in potency.

The dust should be sent to:

Dr. Dan Campbell
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